Genetic structure of Mataco-Guaycurú speakers from Argentina and the extent of their genetic admixture with neighbouring urban populations

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Argentina hosts more than 30 Native American groups, who are widely distributed throughout the country. Mataco-Guaycurú speakers settled in the ecoregion of Gran Chaco and represent 26.7% of the extant aboriginal population of the country. To further investigate the genetic attributes of these speakers, we focused our attention on four aboriginal groups, namely, Wichí, Toba, Pilagá and Mocovi, belonging to the Mataco-Guaycurú linguistic group. Our main goal was to evaluate the interrelationships among the groups and the relationships of these groups with admixed urban populations and to assess correspondences between molecular analysis and historical information. A total of 890 samples (282 Native Americans and 608 inhabitants of admixed urban areas) were analysed. Genetic information was gathered from 15 autosomal STRs, 17 Y-STRs, entire mtDNA control region sequences, 24 AIM-SNPs and 46 AIM-DIPs. Native American signatures were detected in 97.9% of mtDNA lineages, 89.1% of Y-haplotypes and 90.3% to 96.9% of autosomal markers. Wichí exhibited the genetic composition with the largest Native American contribution among the groups and a weak signal of gene flow. This work provides extended genetic information of potential interest in the fields of molecular anthropology and forensic genetics.

The demographic history of Argentina is the result of multiple migration events. Initially, Native American settlers, migrating southward, arrived at the region presently occupied by Argentina over 12,000 years ago. During this period, the migrating tribal groups differentiated by developing unique cultures and languages. The indigenous population settled in this region is estimated to have consisted of 300,000–500,000 people at the time when the first large group of European conquerors arrived. The overall distribution was uneven, with a higher population density in the west, flanking the Andes, and a lower density in the “pampas” and Patagonian regions1. Subsequently, admixture between Native Americans and European conquerors began, followed by Native American admixture with the colonists, resulting in new demographic categories including mestizos (admixed between Native Americans and Europeans), criollos (European descendants born in the Americas), mulatos (admixed between Europeans and Africans, forcibly introduced as slaves since the 17th century), sambos (admixed between Native Americans and Africans) and many others resulting from the admixture between these new categories. Progressively, a new dominant population (consisting mainly of criollos) displaced the autochthonous populations (indigenous inhabitants) to outlying areas. The conquest followed by colonial expansion displaced aboriginals and reduced the geographical areas occupied by them, who consequently experienced a strong reduction in number due to persecution, death and subjugation.

The Gran Chaco (“Chaco” means “hunting territory” in the Quechua language) is a South American eco-geographical region that includes North-Central Argentina, Bolivia, Paraguay and southern Brazil. This extended area is located between latitudes 16°50'S and 33°50'S and between longitudes 67°50'W and 57°50'W,
covering an area of approximately 1,391,000 km². Aboriginal populations inhabiting the Argentinean part of the Chaco region were the last to submit to the power of the Spaniards and then to the national government.

When the Spaniards arrived in Argentinean Chaco in the 16th century, there were three cultural groups: the so-called “typical chaquenses” (Mataco-Mataguayo and Guaycurú); the group in the jungle, immigrating from Amazonas (Tupi-Guarani and Arawak); and the group in the mountains or Andean region (Lule-Vilca)23.

The tribes of Guaycurú speakers, including Toba, Pilagá, Mocoví and Abipón (extinct), became equestrian after the introduction of horses by the Europeans between the 16th and 17th centuries. This novelty, adopted primarily by Toba and Mocoví, facilitated their expansion to the south. Mataco (Wichi) did not incorporate the horse and remained pedestrian in the northwestern region of the Argentinean Gran Chaco.

The Mataco-Guaycurú linguistic branch included twelve languages spoken in Argentina, Bolivia, Brazil and Paraguay. There are no clear relationships between Mataco-Guaycurú languages and other aboriginal languages; some authors related them to Macro-Ge4, while others related them to Macro-Panoan5.

Currently, according to the last Argentinean Supplementary Survey of Indigenous Peoples6, there are 40,036 Wichí (90.3% of the Wichí who inhabit the country) inhabiting Chaco, Formosa and Salta Provinces; 69,452 individuals of Toba ethnicity (68.5%) inhabiting Chaco, Formosan and Santa Fe Provinces; 4,465 Pilagá (88.4%) living in Formosa Province and 15,837 Mocoví (76.7%) living in Chaco and Santa Fe Provinces. In total, these ethnicities constitute approximately 26% of the extant Native American populations inhabiting Argentina.

In this work, we analysed the genetic relationships between different groups of Mataco-Guaycurú-speaking tribesmen. We used a large set of polymorphic genetic markers transmitted either bi- or uniparentally. The marker set included autosomal short tandem repeats (STRs), Y-chromosome STRs, the entire control region of the mitochondrial DNA (mtDNA) D-loop sequence and ancestry-informative markers (AIMs) that included deletion-insertion polymorphisms (AIM-DIPs) and single nucleotide polymorphisms (AIM-SNPs), which were used to obtain the genetic landscape of these tribal groups. To evaluate the strength of the genetic relationships between isolated tribal groups and admixed urban populations, a sample set from Formosa, Chaco, Santa Fe Provinces and the cosmopolitan population of Buenos Aires Province were also analysed. We assessed the extent of gene flow between the populations and the three major parental lineages, namely, the Sub-Saharan African, European and Native American lineages, based on the Centre d’Étude du Poly morphisme Humain (CEPH) Panel datasets.

Previous studies focusing on tribal groups inhabiting Gran Chaco analysed serological polymorphisms2, HLA markers3, mitochondrial Native American haplogroup (hg) frequencies by PCR/RFLP assays4, mtDNA hypervariable region I sequences5, a set of autosomal STRs6 and Y-STRs7. Demarchi and García Ministro5 summarized previous analyses6–8 and concluded that the Argentine population of Gran Chaco was genetically homogeneous, with intense gene flow between the Mataco and Guaycurú groups.

Aiming to broaden the scope of available genetic data, we expanded the analysed groups, the number and type of polymorphic genetic markers used and the extent of mtDNA sequences. The data obtained allowed us to analyse genetic structure and gene flow by STRs and AIMs and provided novel information concerning Y-chromosomal and mtDNA haplotypes. This work provides genetic information that can be used to better characterize the descendants of Native Americans. The large dataset provided here significantly expands existing genetic databases about the region and might be of interest to molecular anthropologists and human population and forensic geneticists.

Results

Admixture analysis. Aiming to investigate the genetic composition of Mataco-Guaycurú speakers as a consequence of internal migrations and after contact with European descendants, we compared genetic information across 15 autosomal STRs, 46 AIM-DIPs and 24 AIM-SNPs to estimate the degree of genetic admixture.

Fifteen autosomal STRs were typed in 70 Pilagá, 54 Wichí, 121 Toba and 37 Mocoví belonging to the Mataco-Guaycurú-speaking group (see Fig. 1 and Materials and methods for details on the samples). Supplementary Material 1-a shows the frequency distributions across loci, molecular indices and Hardy-Weinberg equilibrium (HWE) results as well as a summary of the autosomal STR analysis results.

We included a sample set of reference populations from the CEPH panel (Sub-Saharan African, CEPH-AFR; European, CEPH-EU and Native American, CEPH-NA) and admixed populations from the provinces inhabited by the Native American sample donors: Formosa, Chaco and Santa Fe. A sample from Buenos Aires Province was also included since this region contains the most cosmopolitan population and experienced the highest influx of internal immigration.

Figure 2 summarizes Nei’s genetic distance and the average number of pairwise differences within each population and between them. The Wichí group exhibited the smallest average number of within-population pairwise differences (indicating that this group was the most homogenous, in agreement with this group having the lowest observed heterozygosity) and the lowest between-population differentiation among the Native American groups.

Slatkin’s linearized genetic distances, depicted in the multi-dimensional scaling (MDS) plot shown in Fig. 3a, revealed two clusters, one including Pilagá, Toba and CEPH-NA and another including urban Argentinean populations, closer to CEPH-EU. The Mocoví group was located between the mentioned clusters. The Wichí group exhibited the highest genetic distance from the rest of the Native American groups and the highest genetic distance from the urban and CEPH-EU samples; CEPH-AFR was isolated in the graph. The genetic distances of Mocoví and Wichí were statistically significant with respect to the remaining populations analysed.

AMOVA taking into account the urban population vs Toba (pooled into one group) vs the rest of the Mataco-Guaycurú groups revealed that 6.6% of the variation was among groups (p = 0.00684 +/- 0.00231) and no significant variation among populations within groups (0.11339 +/- 0.00819) (Supplementary Material 1-a).

Genetic structure was assessed with STRUCTURE software using 15 autosomal STRs routinely used for forensic identification purposes. Table 1 summarizes the results, including those for parental populations from the
CEPH panel. Although this panel of autosomal microsatellites underestimated the aboriginal component, there was a correlation between this result and those obtained with AIM-DIPs (analysed in 161 Mataco-Guaycurú samples) and AIM-SNPs (analysed in 103 Mataco-Guaycurú samples) (Supplementary Material 1-b). The AIM-DIP results provided a clear overview of the genetic structure of the populations under study. Wichí exhibited the largest Native American contribution, and Mocoví, the smallest. The Native American component decreased from north to south, i.e., from Wichí to Mocoví. No significant differences in genetic composition were observed among Toba from Formosa, Chaco and Santa Fe. The AIM-SNPs showed a good correlation with data obtained from AIM-DIPs in Wichí, Pilagá and Toba (Fig. 4 and Supplementary Material 1-b).

Analyses of allele frequencies by the centroid method independently confirmed the results described above. Figure 5 shows the amount of gene flow experienced by a population examined by the centroid method. The regression line represents the expected heterozygosity; populations plotted above the line would have received higher-than-average gene flow, while those positioned under the regression line would have been less impacted by gene flow and, therefore, would have evolved in a relatively isolated manner. The positions of the populations relative to the regression line vary widely in the panels in Fig. 5(a–c) because of a reduction in the number of groups included and the number of alleles in each dataset. As expected, Wichí was plotted far below the regression line, indicating that the gene flow experienced was substantially lower than the average computed for the whole set of populations included in the analysis. In contrast, Mocoví was located above the regression line, indicating that this group may have been exposed to more gene flow.
Analysis of Y-STRs. Seventeen Y-STRs and the SNP M3-Q3 were analysed in 210 male samples. Supplementary Material 2 summarizes the haplotype distribution across populations, the molecular diversity indices, the haplotypes shared by groups and a comparative analysis with previously published reports.

A microvariant at locus DYS385b (allele 16.1) was observed in three haplotypes in Pilagá and five haplotypes in Wichí. One of these haplotypes was the most frequent haplotype in Wichí (29.3%) and in the global Mataco-Guaycurú sample. Two of six haplotypes were previously described in the Toba group from northern Chaco Province by Toscanini et al. (Supplementary material 2).

Figure 6 shows a median-joining (MJ) network of Y-STR haplotypes in Mataco-Guaycurú speakers. The network includes several star-like configurations that might indicate recent expansions of founder haplotypes. The majority of the shared Y-haplotypes were shared by Wichí, Toba and Pilagá from Formosa Province.

Table 1. Admixture extent in Mataco-Guaycurú groups analysed by means of 15 autosomal STRs. Parental populations from the CEPH panel: African (AFR), European (EU) and Native American (NA).
An MDS plot of the Q1a3a Y-haplotypes is presented in Fig. 3b. Pilagá clustered with the Toba groups. The genetic distances between Wichí and the other groups were statistically significant. In addition, statistically significant distances were observed between Mocovi and TobaF and between Mocovi and TobaCh (see Supplementary Material 2).
Only 23 of 210 (10.9%) Y-haplotypes were non-Amerindian. The number of non-Amerindian Y-hgs increased from north (Pilagá: 4/72; 5.5%) to south (Mocoví: 10/17; 58.8%).

**Figure 4.** Graphical representation of admixture analysis by STRUCTURE software based on autosomal STRs (a), AIM-DIP (b) and AIM-SNPs (c) (see data in Supplementary Material 2-b). Colour codes: orange represents the African component; green, the European component, and blue, the Native American component. The internal semi-circular bars indicate a gradient from white to black in the north-to-south direction of the urban and Native American populations included in the analysis. Toba from Formosa, Chaco and Santa Fe are presented as the unified group “Toba”.

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A total of 245 haplotypes of the entire mtDNA D-loop region are summarized in Supplementary Material 3 (haplotype dataset, diversity indices, haplotypes shared by groups and the comparative study). Using the hg assignment tool EMPOP, we determined that 97.9% of the haplotypes belonged to Native American hgs. Only five samples exhibited non-Native American signatures. Figure 7 shows hg frequency distributions across groups. Hg B2 is well represented in all groups; hg A2 was the most frequent in Mocoví, while hg C1 was absent in Wichí and Toba from Chaco. Hgs D1 and D4j were detected in different proportions, with very low frequencies in Mocoví.

Genetic distances based on mitochondrial control region sequencing data were statistically significant in most cases, except for those of Toba-Ch with Toba-F and Toba-SF. The highest genetic distances were observed between Mocoví and all other groups (Supplementary Material 3 and Fig. 3c).

Figure 8 depicts the networks between some hgs, including D1j (N = 4), D1e (N = 27), D4j2 (N = 23), D1f (N = 1), B2h (N = 4), B2v (N = 1) and B2o (N = 4). Mataco-Guaycurú haplotypes were related to nodal sequences as well as sequences from the PhyloTree database (http://www.phylotree.org/). Several unique mutations in the D-loop region differentiated the Mataco-Guaycurú sequences.

Sub-hg D1j was found in four samples from Mocoví and Toba but not observed in the EMPOP database. García et al. studied this hg, compiling a list of D1j haplotypes belonging to Argentinian populations, mainly from central areas of the country. In this study, García proposed a local origin of D1j in the Sierras Pampeanas region of Argentina. In addition, this hg was observed in the Mapuche group (Sala and Corach, N = 2/39).
Notably, the haplotypes observed in the present work differed from all previously described haplotypes due to their unique mutations (T538C, T16093C and T16157C).

Sub-hg D1e was observed in 27 samples from the Toba and Wichí groups. Disregarding insertions at 309, one group of 17 samples and another of 7 samples were identical (including private mutations). In addition, three single haplotypes, two with different private mutations and one identical to the nodal haplotype, comprised the D1e set in the Mataco-Guaycurú sample. This sub-hg was observed twice by Bobillo et al.\textsuperscript{18} (in northeastern Argentina) and in the Chiriguano Native American group\textsuperscript{19}, all with different sequences.

Sub-hg D1f was observed in only one sample of Toba from Formosa, with a deletion at position 16,166. This haplotype does not match the other D1f samples previously described by Bobillo et al.\textsuperscript{18} or García et al.\textsuperscript{16}.

Sub-hg D4j2 was observed in 8 Toba samples and was the most frequent hg in Pilagá (15/56). This hg was observed in one sample from southern Argentina\textsuperscript{18} but has not been observed in any other aboriginal group previously investigated by our team: Mapuche and Tehuelche\textsuperscript{17}, Chiriguano\textsuperscript{19} or Mbýá - Guarani\textsuperscript{20}.

Regarding sub-hgs belonging to the B2 branch (Fig. 8a), we found sub-hg B2h in 4 Pilagá samples, B2v in one Wichí sample and B2o in 4 Tobas samples. These sequences represent unique mutations that were not found in other Native American groups studied by our team or in the EMPOP database. The sub-hgs B2o and B2h were described previously in Argentina (B2o in 3 samples from Jujuy, northern Argentina, by Cardoso et al.\textsuperscript{21}; B2h in 2 samples from Buenos Aires by Bobillo et al.\textsuperscript{18}).

Other sub-hgs, including A2ag (in one Mocoví) and A2ah (in one Toba), were not found or were infrequent in other Native American groups from Argentina (A2ah was observed once in Chiriguano by Sala et al.\textsuperscript{19}).

Discussion

Genetic data emerging from this study of extant Mataco-Guaycurú-speaking groups inhabiting the ecoregion of “Gran Chaco” in Northern Argentina allowed us to gather novel information that broadens the scope of present-day knowledge about Mataco-Guaycurú speakers.

In agreement with the conclusion of Demarchi et al.\textsuperscript{13}, no statistically significant differences were found between the Toba groups that inhabit different provinces. This result might reflect a homogeneous group due, in part, to the fact that the Toba people speak a common language (Qom) and to recent migration events that led to their present-day dwellings.

In clear contrast with the homogeneity of Toba, the other groups showed peculiar attributes concerning genetic structure, gene flow and uniparentally transmitted markers.

As expected, and based on historical records, the gene pool of Wichí contained the largest aboriginal component among the groups; this group was the most genetically distant from the other groups. Isolated conditions were reflected in all the polymorphic genetic markers tested and underscored by the centroid method, revealing the lowest gene flow among the aboriginal groups included herein. In addition, the smallest allele size range and number of alleles per locus for all the studied loci were observed in Wichí. The hypothesis that a recent bottleneck can still be detected in these groups is being evaluated by expanding the number of DNA markers (manuscript in preparation).

The Native American component decreased from north to south, i.e., from Wichí to Mocoví. This pattern can be explained by the higher internal migration experienced by Toba and Mocoví because of the incorporation of the horse as a tool of power and conquest during colonial times. In contrast to these groups, Wichí remained pedestrian in the northern range of their distribution, in Formosa and Salta Provinces, with reduced contact with other groups.

Currently, the natural environment is severely affected by intense farming and highly technical agricultural practices, forcing Native American descendants to migrate to urban areas to search for work opportunities. Table 2 summarizes the aboriginal composition of the groups analysed.

Demarchi and Mitchell\textsuperscript{12} reported that 52.6% (10/19) of Pilagá sample donors carried a non-Native American-specific Y-hg. In contrast, we found that only 5.5% (4/72) exhibited the non-Amerindian signature on the Y chromosome. This difference may be due to a difference in sample sizes and/or sampling sites (see the

Figure 7. Entire mitochondrial D-loop sequence-based hg frequency distributions for Mataco-Guaycurú speakers from Argentina.
Figure 8. MJ network of hg B2 (a) and hgs D1 and D4 (b), connecting Mataco-Guaycurú haplotypes with nodal (indicated with an arrow) and PhyloTree-associated sequences (accession numbers are also indicated). The analysis was restricted to D-loop sequences, disregarding insertions at position 309. The deletion at 16,166 was weighted three times over transitions. Private mutations are indicated in bold. Colour codes are included in the figure.

Table 2. Bi- and uniparental aboriginal compositions of Mataco-Guaycurú speakers included in this study.

| Province | Ethnic group | Native American component | Demographic information |
|----------|--------------|---------------------------|-------------------------|
|          |              | Autosomal AIM-DIP | Y-chromosome | mtDNA | Population per ethnicity/ total population per province (%) |
| Wichí    |              | 96.9%        | 100%        | 100%  | 2.97%          |
| Formosa  | Pilagá       | 96.1%        | 94.5%       | 100%  | 0.89%          |
| TobaF    |              | 94.9%        | 91.7%       | 100%  | 2.51%          |
| Chaco    | TobaCh       | 94.9%        | 100%        | 88.9% | 3.12%          |
| Santa Fe | TobaSF       | 94.1%        | 77.8%       | 96%   | 0.47%          |
| Mocovi   |              | 90.3%        | 41.2%       | 100%  | 0.45%          |
comparative analysis in Supplementary Material 2). Such a discrepancy substantially affects the overall genetic landscape obtained as well as the reconstructed relationships of Pilagá with other ethnic groups. Although Pilagá did not undergo strong migratory movements, they had more interactions with Toba, possibly explained by their linguistic affinity; since both groups belong to the Guaycurú linguistic sub-branch, and their geographical proximity; both factors might promote a certain degree of gene flow. These patterns were reflected in the genetic distances between the Pilagá and Toba groups (p > 0.05) based on both autosomal STRs and Y-STRs.

Microvariant DYS385 16.1 was observed in six different haplotypes in Pilagá and Wichi, but we did not find it in the Toba groups, as did Toscanini15. Network configuration showed a possible founder haplotype carrying this variant and connecting the three ethnicities (HY1 haplotype, in Supplementary Material 2).

The statistically significant mitochondrial genetic distances between most of the analysed groups indicate less migration of women than of men, compatible with the hypothesis of matrilocality.

Cabana et al.10 reported results of mtDNA analysis of Pilagá, Toba and Wichi ethnicities based on hypervariable region I, with N = 204. These authors found that 48/204 (23.5%) haplotypes differed, with 31% shared by different Mataco-Guaycurú-speaking groups. The additional genetic information provided herein (entire D-loop sequencing data) allowed us to increase the information about this marker, such as the haplotype diversity and haplotype discrimination power within each hg (see the comparative analysis in Supplementary Material 3). The haplotype distribution reported here is similar to that reported by Cabana, except for the absence of hg C in Wichi and in Toba from Chaco (present in Cabana’s study). These results could be explained by a difference in sample sizes or sampling locations (in Cabana’s publication, the sampled geographical location was not clear).

Recently published work on the Alto Paraná region of Paraguay23 reported some of the abovementioned hgs (B2b3a, 2%; B2o, 1%; B2h, 4%; D1e 4% and D1f, 1%), although the D-loop haplotype sequences were different from those of Mataco-Guaycurú reported in this study.

Based on autosomal and patrilineal compositions (55.5% of Y-haplotypes were non-Amerindian), Mocóvi were the most admixed group. Furthermore, genetic distance representation via MDS plots (Fig. 3a) placed the Mocoví group between the aboriginal and urban populations. This observation was also reflected in the extent of gene flow, as shown in Fig. 5c, where only the Mocoví group was placed above the regression line. However, Native American hg signatures in maternal mtDNA were present in 100% of the analysed Mocoví, indicating clear bias in the admixture process, as reflected for most areas of the country and the continent.

Statistical information obtained from population genetics analysis is presented in the supplementary information since it might be of interest in molecular anthropology as well as for forensic purposes. Some Y-STRs and mtDNA haplotypes found in the analysed populations have been uploaded to specific databases, such as the Y-chromosome Haplotype Reference Database (YHRD) and EMPOP. Further sequencing of the full mitochondrial genome could expand knowledge of the sub-hg diversity of the aboriginal populations investigated.

This work complements previous research carried out by other groups and by our team, who have worked on the genetic characterization of different aboriginal groups inhabiting Argentina and whose main goal is to increase knowledge about the native populations that persist despite the multi-ethnic admixture process taking place in present-day Argentina.

Materials and Methods

Individuals. Sample donors read and signed a written informed consent statement. Research projects and consent statements were approved by the Bioethics Committee of the School of Pharmacy and Biochemistry, Buenos Aires University, Argentina (Res. 1053, Expte 744085/FFyB-UBA). The research was performed in accordance with relevant guidelines, and signed informed consent was obtained from all donors. A set of 890 individuals was analysed, including 282 Native American tribesmen who spoke Mataco-Guaycurú and inhabited three Argentinean provinces (Formosa, Chaco and Santa Fe), 608 individuals from urban populations of four provinces (the abovementioned provinces and Buenos Aires Province), and three parental populations from the CEPH panel. Table 3 provides a summary of population codes, ethnicities, geographical locations, provinces and sample sizes. Figure 1 depicts the geographic location of the sampling sites.

Analytical methods. DNA extraction. DNA was extracted from either blood samples or liquid saliva by using conventional protocols23,24.

Autosomal STRs. We typed Amelogenin and fifteen STRs: D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, VWA, D8S1179, TPOX, and FGA. Experimental procedures and analyses were performed according to the manufacturer’s protocol (PowerPlex 16 System kit, Promega Corp., Madison, USA).

Y-chromosome STRs and Y-SNPs. We analysed seventeen Y-STRs included in the AmpFlSTR YFiler Kit (Thermo-Fisher, Foster City, USA) in all the samples, except in TobaSF and Mocoví samples, which were analysed with twelve Y-STRs included in the PowerPlexY kit (Promega Corp.).

The haplotypes included in this study have been deposited in the YHRD (http://www.yhrd.org; accession numbers: YA003004, YA003005, YA003802, YA002989, YA004639 and YA004640, available in the next update- R62-).

The Y-SNP M3-C/T (Q1a3a1 hg) was analysed by real-time PCR followed by high-resolution melting analysis as previously described25.

AIM-DIP and AIM-SNP analyses. A total of 307 reference samples from the CEPH panel, 161 samples from Mataco-Guaycurú speakers (31 Wichí, 41 Pilagá, 57 Toba and 32 Mocoví) and 200 samples from the urban populations were typed with a panel of 46 AIM-DIPs. In addition, 247 reference samples, 103 Mataco-Guaycurú samples and 162 urban population samples were analysed with 24 autosomal SNPs (AIM-SNPs). A total of 46
Aim-DIPs and 24 AIM-SNPs were amplified as previously described. The analysed samples as well as results from the structure analysis are summarized in Supplementary Material 1b. Given the homogeneous genetic structure observed in Toba from Formosa, Chaco and Santa Fe, the data were treated as a single group called Toba.

mtDNA D-loop sequencing. We sequenced the entire mtDNA D-loop (1,120 base pairs). Amplification, purification and electrophoresis conditions were as previously described. All sequencing analyses were performed with both forward and reverse primers (with at least four to six primers for each sample). Electropherograms were performed using the distance from the centroid (ri) were created in Microsoft Excel 2016. Regression plots reflecting the proportion of heterozygotes (H)

Table 3. Sample codes, ethnic group names, sampling sites, geographic locations, provinces and sample numbers analysed in the present work. *N: Number of unrelated individuals.

| Pop. code | Ethnicity | Geographic location | Province | N* |
|-----------|-----------|---------------------|----------|----|
| Pilagá    | Pilagá    | Localities: Colonia Ibarreta, 25°13'05"S, 59°51'05"W and Las Lomitas, 24°42'26"S, 60°35'40"W | Formosa  | 72 |
| Wichí     | Wichí     | Localities: Ingeniero Juarez, 25°10'06"S, 58°7'60"W and El Yacaré, 23°38'42"S, 62°15'01"W | Formosa  | 54 |
| TobaF     | Toba      | Localities: Laguna Blanca, 25°07'46"S, 58°14'70"W | Formosa  | 38 |
| TobaCh    | Toba      | Localities: General Jose de San Martin, 26°32'22"S, 59°20'32"W | Chaco    | 26 |
| TobasF    | Toba      | Localities: Rosario, 32°55'17"S, 60°39'84"W and Santa Fe Capital, 31°35'47"S, 60°41'59"W | Santa Fe | 57 |
| Mocovi    | Mocovi    | Localities: Tostado, 29°13'84"S, 61°46'10"W | Santa Fe | 37 |
| FOR       | Admixed   | Urban area, 26°11'55"S, 58°10'33"W | Formosa  | 147 |
| CHA       | Admixed   | Urban area, 27°27'5"S, 58°59'12"W | Chaco     | 118 |
| SFE       | Admixed   | Urban area, 31°38'0"S, 60°42'0"W | Santa Fe  | 178 |
| BA        | Admixed   | Urban areas, 34°35'59"S, 58°22'55"W | Buenos Aires | 165 |
| CEPH-AFR  | African   | Bantu Kenya, Biaka Pygmy, Mandenka, Mbuti Pygmy, San and Yoruba | 70 |
| CEPH-EU   | European  | Basque, French, Italian, Sardinian, Orcadian, Russian and Tuscan | 84 |
| CEPH-NA   | Native American | Colombiano, Karitiana, Surui, Maya and Pima | 50 |

Table 4. Summary of markers analysed in each Mataco-Guayurú group.

| Markers          | Wichí | Pilagá | Toba | Mocovi |
|------------------|-------|--------|------|--------|
| Autosomal STR    | 54    | 70     | 121  | 37     |
| AIM-DIP          | 31    | 41     | 57   | 32     |
| AIM-SNP          | 51    | 26     | 26   | —      |
| Y-STR + Y-SNP    | 40    | 72     | 81   | 17     |
| mtDNA seq.       | 48    | 56     | 101  | 27     |

Statistical analysis. Allele frequencies, HWE, gene and haplotype diversities, genetic distances and heterozygosity were determined with Arlequin v3.5.2.2.36. Admixture analysis was performed with STRUCTURE v2.3.4 software, employing 15 autosomal STRs, 24 AIM-SNPs and 46 AIM-DIPs. Genotypes of the parental populations, including the Sub-Saharan African, European and Native American populations, were obtained from published data derived from CEPH Panel samples. For STRUCTURE analysis, five iteration rounds were used. The number of parental populations (k) was set from two to five. Monte Carlo–Markov chain simulation, including a burn-in step of 10,000 iterations followed by 20,000 iterations for data gathering, was performed for each round. The number of populations assumed was initially set to the number of parental populations. An admixture model and independent allele frequencies were used. The most likely value for the number of parental populations (k = 3) was determined using the online program Structure Harvester, which enables implementation of the Evanno method. Further data analysis was performed using CLUMPP. Graphical representation was performed with Ancestry Painter software (http://www.picb.ac.cn/PGG/resource.php; 34).
distributions. Y-Hgs were inferred using Whit Athey's Haplogroup Predictor (http://www.hprg.com/hapest5/index.html), and Native American specific Hg was confirmed with the SNP M3-Q3. A matrix of normalized Slatkin's genetic distances was represented as an MDS plot using XLSTAT (Addinsoft Corp) software.

MJ networks were obtained with Network 5.0.1.1 software (http://www.fluxus-engineering.com/). Y-STR mutations were weighted according to Qamar et al.24

Data availability
The datasets analysed during the current study are available from the corresponding author upon reasonable request or are included in this published article (and its supplementary information files).

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References
1. Martinez Sarasola, C. Nuestros paisanos los indios. (Emece. Eds, Buenos Aires, Argentina, 2005).
2. Altamirano, M. A., Shardiella, C. R. & Dellama de Pietro, A. N. Historia del Chaco. (Editorial Cosmos, Argentina, 1994).
3. Kersten, L. Las tribus indígenas del Gran Chaco hasta fines del Siglo XVIII: una contribución a la etnografía histórica de Sudamérica. (Editorial Leiden, https://rdu.unc.edu.ar/bitstream, Universidad Nacional del Nordeste, Argentina, 1968).
4. Viegas Barros, J. P. La hipótesis de parentesco Guaicurú-Mataguayo: estado actual de la cuestión. Revista Brasileira de Linguística Antropológica 2, 333–338 (2013).
5. Greenberg, J. & Ruhlen, M. An Amerind Etymological Dictionary. Stanford: Dept. of Anthropological Sciences (Stanford University Press, 2007).
6. ECPI (2004–2005) ECPI, Encuesta Complementaria de Pueblos Indígenas. Instituto de Estadísticas y Censos, Buenos Aires, https://www.indec.gob.ar/.
7. Goicoechea, A. S. et al. Genetic relationships between Amerindian populations of Argentina. Am J Phys Anthropol 115:133–143 (2001).
8. Dejean, C. B., Crouau-Roy, B., Goicoechea, A. S., Avena, S. A. & Carnese, F. R. Genetic variability in Amerindian populations of Northern Argentina. Genetics and Molecular Biology 27, 489–495 (2004).
9. Demarchi, D. A., Panzetta-Dutari, G. M. & Motran, C. C. López de Basualdo, M. A., Marcellino, A. I. Mitochondrial DNA haplogroups in Amerindian populations from the Gran Chaco. Am J Phys Anthropol 113(3), 199–203 (2001).
10. Cabana, G. S., Merrirwether, A., Hunley, K. & Demarchi, D. A. Is the Genetic Structure of Gran Chaco Populations Unique? Regional Perspectives on Native South American Mitochondrial DNA Variation. Am J Phys Anthropol 131, 108–119 (2006).
11. Crossetti, S. G. et al. Autosomal STR genetic variability in the Gran Chaco native population: Homogeneity or heterogeneity? Am J Hum Biol 20(6), 704–11, https://doi.org/10.1002/ajhb.20798 (2008).
12. Demarchi, D. & Mitchell, R. J. Genetic Structure and Gene Flow in Gran Chaco Populations of Argentina: Evidence from Y-Chromosome Markers. Human Biology 76(3), 413–429 (2004).
13. Demarchi, D. A. & García Ministro, A. Genetic Structure of Native Populations from the Gran Chaco Region, South America. Int J Hum Genet 8(1–2), 131–141 (2008).
14. Harpending, H. C. & Ward, R. H. Chemical systematics and human populations. In Biochemical aspects of evolutionary biology (M Nilscki, ed.). Chicago: University of Chicago. 213–256 (1982).
15. Toscanini, U. et al. Male Lineages in South American Native Groups: Evidence of M19 Traveling South. Am J Phys Anthropol 146, 188–196 (2011).
16. García, A., Pauro, M., Nores, R., Bravi, C. M. & Demarchi, D. A. Phylogeography of mitochondrial haplogroup D1: an early spread of subhaplogroup D1b from Central Argentina. Am J Phys Anthropol 149(4), 583–590, https://doi.org/10.1002/ajpa.22174 (2012).
17. Salas, A. & Corach, D. Analysis of admixture and genetic structure of two Native American groups of Southern Argentinean Patagonia. Mol Biol Rep 41, 1533–1543 (2014).
18. Bobillo, M. C. et al. Amerindian Mitochondrial DNA Haplogroups Predominate in the Population of Argentina: towards a first nationwide mitochondrial DNA sequence database. Int J Legal Med 124, 263–268 (2010).
19. Sala, A. et al. Historical records under the genetic evidence: “Chiriguano” tribe genesis as a test case. Mol Biol Rep. 45(5), 987–1000, https://doi.org/10.1007/s11033-018-4234-0 (2018).
20. Sala, A. et al. Genetic analysis of six communities of Mbya-Guaraní inhabiting North Eastern Argentina by means of nuclear and mitochondrial polymorphic markers. Hum Biol 82, 433–456 (2010).
21. Cardoso, S. et al. Mitochondrial DNA control region data reveal high prevalence of Native American lineages in Jujuy province, NW Argentina. Forensic Sci Int Genet 7(3), e52–5, https://doi.org/10.1016/j.fsigen.2013.01.007 (2013).
22. Simon, F. et al. The maternal inheritance of Alto Paraná revealed by full mitochondome sequences. Forensic Sci Int Genet. 39, 66–72, https://doi.org/10.1016/j.fsigen.2018.12.007. (2019).
23. Sambrook, J., Fritsch, E. F. & Maniatis, T. Molecular cloning: A laboratory manual. (2nd ed.). Cold spring harbor laboratory, New York, U.S.A 1989.
24. Quinque, D., Kittler, R., Kayser, M., Stoneking, M. & Nasidze, I. Evaluation of saliva as a source of DNA for population and association studies. Anal Biochem 15, 272–277 (2006).
25. Zuccarelli, G. et al. Rapid screening for Native American mitochondrial and Y-chromosome haplogroups detection in routine DNA analysis. Forensic Sci Int Genet 5, 105–108 (2011).
26. Pereira, R. et al. Straightforward Inference of Ancestry and Admixture Proportions through Ancestry-Informative Insertion Deletion Multiplexing. PLoS ONE 7(1), e29684, https://doi.org/10.1371/journal.pone.0029684 (2012).
27. Lao, O., van Duijn, K., Kersbergen, P., de Knijff, P. & Kayser, M. Proportioning whole-genome single-nucleotide polymorphism diversity for the identification of geographic population structure and genetic ancestry. Am J Hum Genet 78, 680–690 (2006).
28. Parson, W. & Dür, A. EMPO: a forensic mtDNA database. Forensic Sci Int Genet 1, 88–92 (2007).
29. Excoffier, L., Laval, G. & Schneider, S. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1, 47–50 (2005).
30. Prichard, J. K., Stephens, M. & Donnelly, P. Inference of population structure using multilocus genotype data. Genetics 155, 945–959 (2000).
31. Salazar-Flores, J. et al. Admixture and genetic relationships of Mexican Mestizos regarding Latin American and Caribbean populations based on 13 CODIS-STRs. Homo. 66(1), 44–59, https://doi.org/10.1016/j.jchb.2014.08.005 (2015).
32. Earl, D. A. & von Holdt, B. M. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4, 359–361 (2012).
33. Jakobsson, M. & Rosenberg, N. A. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23, 1801–1806 (2007).
34. Feng, Q., Lu, D. & Xu, S. AncestryPainter: A Graphic Program for Displaying Ancestry Composition of Populations and Individuals. Genomics Proteomics Bioinformatics 16(5), 382–385, https://doi.org/10.1016/j.gpb.2018.05.002 (2018).
35. Nei, M. Molecular Evolutionary Genetics. New York: Columbia University Press (1987).
36. Kayser, M. et al. Evaluation of Y-chromosomal STRs: a multicenter study. *Int J Legal Med* **110**, 125–133 (1997).
37. Bandelt, H. J., Forster, P. & Röhl, A. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* **16**, 37–48 (1999).
38. Qamar, R. et al. QY-chromosomal DNA variation in Pakistan. *Am J Hum Genet.* **70**(5), 1107–1124, https://doi.org/10.1086/339929 (2002).

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**Author contributions**
Study design: A.S. and D.C.; experimental work: A.S.; statistical analysis: A.S., M.C. and D.C.; initial manuscript draft: A.S.; revisions: D.C. and A.S. All authors reviewed the manuscript.

**Competing interests**
The authors declare no competing interests.

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