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Fine-scale genomic analyses of admixed individuals reveal unrecognized genetic ancestry components in Argentina

Native American, African and European genetic ancestries in Argentina

Pierre Luisi*[,1], Angelina García[1,2,3], Juan Manuel Berros[4], Josefina Motti[5], Darío Demarchi[1,2,3], Emma Alfaro[6,7], Eliana Aquilano[8], Carina Argüelles[9,10], Sergio Avena[11,12], Graciela Bailliet[8], Julieta Beltramo[8,13], Claudio M. Bravi[8], Mariela Cuello[8], Cristina Dejean[11,12], José Edgardo Dipierri[7], Laura S. Jurado Medina[8], José Luis Lanata[14], Marina Muzzio[8], María Laura Parolin[15], Maia Pauro[1,2,3], Paula B. Paz Sepúlveda[8], Daniela Rodríguez Golpe[8], María Rita Santos[8], Marisol Schwab[8], Natalia Silvero[8], Jeremias Zubrzycki[16], Virginia Ramallo[17], Hernán Dopazo*[,18,19]

1. Universidad Nacional de Córdoba, Facultad de Filosofía y Humanidades, Departamento de Antropología, Argentina

2. Instituto de Antropología de Córdoba (IDACOR), Consejo Nacional de Investigaciones Científicas y Técnicas – Universidad Nacional de Córdoba, Córdoba, Argentina

3. Universidad Nacional de Córdoba, Facultad de Filosofía y Humanidades, Museo de Antropología, Córdoba, Argentina.

4. Consejo Nacional de Investigaciones Científicas y Técnicas – Laboratorio de Análisis de Datos, Biocódices S.A, Buenos Aires, Argentina

5. Núcleo de Estudios Interdisciplinarios de Poblaciones Humanas de Patagonia Austral (NEIPHA), Consejo Nacional de Investigaciones Científicas y Técnicas – Universidad Nacional del Centro de la Provincia de Buenos Aires, Quequén, Argentina.

6. Instituto de Ecorregiones Andinas (INECOA), Consejo Nacional de Investigaciones Científicas y Técnicas – Universidad Nacional de Jujuy, Argentina

7. Instituto de Biología de la Altura, Universidad Nacional de Jujuy, Argentina

8. Instituto Multidisciplinario de Biología Celular (IMBICE), Consejo Nacional de Investigaciones Científicas y Técnicas – Comisión de Investigaciones Científicas – Universidad Nacional de La Plata, Argentina

9. Grupo de Investigación en Genética Aplicada (GIGA), Facultad de Ciencias Exactas, Químicas y Naturales, Departamento de Genética, Instituto de Biología Subtropical

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(IBS) - Nodo Posadas, Universidad Nacional de Misiones (UNaM) – Consejo Nacional de Investigaciones Científicas y Técnicas, Posadas, Argentina.

10. Cátedra de Biología Molecular. Carrera de Medicina. Facultad de Ciencias de la Salud. Universidad Católica de las Misiones (UCAMI), Posadas, Argentina.

11. Instituto de Ciencias Antropológicas (ICA), Facultad de Filosofía y Letras, Universidad de Buenos Aires, Argentina

12. Centro de Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico (CEBBAD), Universidad Maimónides, Buenos Aires, Argentina,

13. Laboratorio de Análisis Comparativo de ADN, Corte Suprema de Justicia de la Provincia de Buenos Aires, La Plata, Argentina

14. Instituto de Investigaciones en Diversidad Cultural y Procesos de Cambio (IIDyPCa), Consejo Nacional de Investigaciones Científicas y Técnicas – Universidad Nacional de Río Negro, San Carlos de Bariloche, Argentina

15. Instituto de Diversidad y Evolución Austral (IDEAus), Consejo Nacional de Investigaciones Científicas y Técnicas – Centro Nacional Patagónico, Puerto Madryn, Argentina

16. Laboratorio de Genómica. Biocódices S.A., Buenos Aires, Argentina

17. Instituto Patagónico de Ciencias Sociales y Humanas (IPCSH) – Consejo Nacional de Investigaciones Científicas y Técnicas - Centro Nacional Patagónico, Puerto Madryn, Argentina

18. Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

19. Consejo Nacional de Investigaciones Científicas y Técnicas – Biocodices S.A., Buenos Aires, Argentina

* Corresponding Authors: pierrespc@gmail.com, dopazoh@gmail.com

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1 Abstract

We are at the dawn of the efforts to describe and understand the origins of genetic diversity in Argentina from high-throughput data. This knowledge is a primary step in the intent of deciphering the specific genetic bases of diseases and drug response in the country. Similarly to other populations across the Americas, genetic ancestry in Argentinean populations traces back into African, European and Native American ancestors, reflecting a complex demographic history with multiple migration and admixture events in pre- and post-colonial times. However, little is known about the sub-continental origins of these three main ancestries. We present new high-throughput genotyping data for 87 admixed individuals across Argentina. This data was combined to previously published data for admixed individuals in the region and then compared to different reference panels specifically built to run population structure analyses at a sub-continental level. Concerning the European and African ancestries, we confirmed previous results about their main origins, and we provide new insights into the presence of other origins that reflect historical records. As for the Native American ancestry, leveraging genotype data for archaeological samples in the region in order to gain temporal depth in our analyses, we could identify four Native American components segregating in modern Argentinean populations. Three of them are also found in modern South American populations and are specifically represented in Central Chile/Patagonia, Lowlands and Central Andes geographic areas. The fourth one may be specific to the Central Western region of Argentina. Identifying such component has not been straightforward since it is not well

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represented in any genomic data from the literature. Altogether, we provide useful insights into the multiple population groups from different continents that have contributed to present-days genetic diversity in Argentina. We encourage the generation of massive genotype data locally to further describe the genetic structure in Argentina.

2 Author Summary

The human genetic diversity in Argentina reflects demographic mechanisms during which the European colonists invaded a territory where Native American populations were settled. During colonial period, the slave trade also prompted many African people to move to Argentina. Little is known about the origins of the Native American and African components in Argentinean populations nowadays. Genotyping data for 87 admixed individuals throughout Argentina was generated and data from the literature was re-analyzed to shed light on this question. We confirmed that most of the European genetic ancestry comes from the South, although several individuals are related to Northern Europeans. We found that African origins in Argentina trace back from different regions. As for the Native American ancestry, we identified that it can be divided into four main components that correspond to Central Chile/Patagonia, Lowlands, Central Andes and Central Western region of Argentina. In order to understand the specificity of the genetic diversity in Argentina, we should not rely on knowledge generated in other populations. Instead, more effort is required to generate specific massive genomic knowledge at the local level.

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3 Introduction

Natural selection, genetic drift, migrations and admixture shape the genetic composition of human populations. In order to decipher the demographic history of populations and get insights into their specific genetic bases of diseases and drug response, it is primordial to first characterize their geographical genetic structure [1]. Much effort has been made at broad scales in South America. In this article, we present analyses at much finer scales for Argentina.

The first systematic investigation of human genetic variation in the country focused on a limited number markers either uniparental (mtDNA, Y-STRs, Y-SNP; [2–11] or autosomal (Short Tandem Repeats, Ancestry Informative Markers, Alu sequences, indels, and blood groups [12–17]). Studies based on autosomal markers identified an important inter-individual heterogeneity for the African, Native American and European genetic ancestry proportions [12–17]. Accordingly, most of the studies based on uniparental markers showed large differences in the genetic composition of Argentinean populations, accounting for the different demographic histories within the country [2,3,18–21,4–10,17]. A recently published book compiles most of these studies and others to gives a general view about the admixture in Argentinean populations [22]. Although the idea of a 'white' country with most of inhabitant's descendants from European immigrants has now been rejected by these studies, the Argentine founding myth of a white and European nation remains perceptible today [23].

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There is a wealth of information about the European ancestors, both from familial stories and from historical records. On the contrary, little is known about the African and Native American populations that contributed to the admixture events between the continental components. During the great wave of European immigration between the mid-19th to mid-20th centuries, people from Southern Europe (mainly Italy and Spain) settled in Argentina, along with migrants from Western Europe (mainly French), Central Europe (mainly German) and Northeastern Europe (mainly Poles, Ukrainians, Russians) [24]. Historical records attest that immigration waves from Southern Europe were predominant from this continent [24].

The presence of human groups of African origins in Argentina traces back to the slave trade that took place during colonial era, most of the time illegally, and is therefore poorly documented in historical records [25]. It is even very difficult to estimate how many African slaves were brought into current Argentinean territory. Moreover, most of the European people at that time were unaware of African societies with which they traded. Thus, the slaves were ethnically identified according to the last ship they boarded before travelling to the Americas [25,26]. However, this is a poor proxy to infer their actual origins since captains often had to dock in different harbors along the African coasts in order to complete the human load. In addition, many slaves first arrived in Brazil and then were taken to the Río del Plata harbor to be finally distributed across the current Argentinean territory [25]. The latest Brazilian harbor is often the only source of origin information available. In addition, extrapolate the historical records from the entrance point to

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the whole Argentinean territory is a hazardous task since the slaves origin and destination have been changing during colonial era according to varying economical interests and political situations [26,27]. Even then, historical records about the arrival flows of African slaves to Río de la Plata show that Luanda, in current Angola territory in Central Western region of Africa, was the main departure harbor, followed by harbors located in the Gulf of Guinean and on the coast of the present-day Senegalese, Gambian and Sierra Leone territories. The coasts of Indian Ocean, in the current territory of Mozambique, were also an important departure location [27–29].

As for the Native American component, it is difficult to study its origin focusing on present-day communities since their organization has changed drastically from the 16th century, and the arrival of the first conquerors, until the beginning of the 20th century [23]. During the period of conquest and colonization, wars, diseases and forced labor decimated the Native populations. The system of colonial exploitation also often meant the relocation of individuals, families, and communities [30]. Then, the expansion of the nation-state by the late 19th Century can be described as a territorial annexation process and subjugation of the indigenous peoples perpetrated by the Argentinean national armed forces between 1876 and 1917 [31]. For example, the miscalled Conquest of the Desert in the 1870s to integrate Patagonia into the Argentinean state caused that the indigenous populations were subjected to practices of extermination, deportations and social fragmentation [32]. Moreover, the arbitrariness of several academic, historical, economic and political

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factors have greatly influenced the classification of the Native American groups settled in the Argentinean territory

Recently, two articles presented high-throughput genotyping data for hundreds of thousands of autosomal markers through the genome for modern Argentine individuals, and provided the first inferences of which populations contributed to the admixture events among the three main continental components [33,34]. Homburger *et al.* studied 437 admixed individuals from urban centers in 5 South American countries: Argentina, Chile, Colombia, Ecuador and Peru. On the other hand, Muzzio *et al.* obtained genotype data from nearly 400 individuals from 12 populations in Northern and Central Argentina using Illumina exome-based array technology [34]. In both studies, it was found that the European ancestry in Argentina is mainly explained by Italian and Iberian ancestry components according to the historical records. Homburger *et al.* observed a strong gradient in Native American ancestry of South American Latinos between Andean and other South American Native American populations. This gradient is associated with the country of origin and geography of local indigenous populations [33]. In addition, Muzzio *et al.* found that African ancestry is explained by a Western and a Bantu-influenced components and described a Native American component not represented in the 1000 Genomes project (1KGP) [34].

Despite the progress in studying the genetic diversity of the Argentinean populations, the challenge of achieving a fine-scale knowledge has inevitably been scarce. In this context, studies of a wider region, namely South America, can provide important insights into the Native American specific genetic diversity in Argentina

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and its origins. Ancient DNA analyses of the peopling of the Americas suggest that Native American populations of South America descend from two streams from Northern America: one mainly present in the Andes and another one present elsewhere [35]. These streams have replaced the first people settled in the region, whose ancestry was related to the Clovis culture [35]. Genetic continuity for the Native American component appears to have prevailed in the region ever since these replacements [35–37]. However, little is known about the legacy in modern populations of the different ancestries associated to the several waves of population arrivals in South America. Modern Native American populations in the Southern Cone of South America seems to be divided between a Lowlands component and another component that includes Andean, Central Chile and Patagonian populations [38]. A recent genomic study of ancient and modern populations from Central Chile and Western Patagonia further identified that they are differentiated from the Andean and Lowlands populations, supporting a differentiation process throughout South America [37]. In another recent genomic study of modern samples, in which the Southern Cone is only represented by Gran Chaco populations, it has been described a likely common lineage for non-Andean South Americans populations which do probably not share with the Andeans any common ancestor from Central America [39].This supports the hypothesis of many migrations back to Central America different from the Andeans ancestors [39]. It has also been described that the genetic interactions between the peopling routes on both sides of the Andes were limited [39,40].

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The Native American specific genetic diversity in South America has been studied focusing almost exclusively on indigenous communities. In many regions of Argentina, particularly were the Spanish conquerors settled (in the Northwest, Central and Central Western regions), indigenous communities did not maintain their traditional lifestyle. They have rather been incorporated as a workforce in the colonial society. The sedentary life of these communities at the moment of contact partially contributed to this phenomena [23]. The early assimilation to the colonial society made gradually indigenous identity invisible since the communities were then referred to as peasants [41,42]. It implied a cultural annihilation and several Argentinean regions were considered "Indian free" in the mid-20st century [43]. However, the cultural incorporation did not necessarily imply a biological extinction. We argue that focusing only on indigenous communities by "implementing accurate sampling strategies and of selecting representative populations based on historical/linguistic and anthropological information", as advocated by Gnecchi-Ruscone *et al.* [39] for example, is not sufficient to decipher the pre-Columbian history of Native Americans. Although studies based on such sampling strategies [37–39] provide decisive information to understand the evolutionary history of Native American ancestry [22], it implies to disregard many regions of the continent. This is particularly the case of Argentinean territory where only the extreme North and South, separated by ~ 3,700 kilometers, have been studied under this scope. Therefore, alternative strategies must be considered to fill this gap in the intent of describing the Native American ancestry.

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Here, we carry out a fine-scale population genomic study to get insight into the genetic structure and the complex origins of the African, European and Native American ancestries in Argentina populations. Using Affymetrix, Axiom-Lat1 array technology, we genotyped 87 admixed individuals nationwide for more than 800,000 SNPs covering coding and non-coding regions of the genome. Additionally, a dataset with close to 500 individuals from modern and ancient populations throughout South America was constructed. We also pulled together genotype data for over 1,600 individuals from some ~50 Sub-Saharan African populations from the literature. The new data generated in the present study, compared to those data sets, allowed to broaden the knowledge of the sub-continental origins of the three main genetic ancestries of Argentinean populations.

4 Results and Discussion

High-throughput genotyping data was generated for 87 admixed individuals throughout Argentina (**Fig 1** and **S1 Table**). For clarity in the visualization of the results, we used Administrative Regions to classify the admixed individuals analyzed as shown in **Fig 1**. This classification has not been used for any statistical analyses. The generated data was compared to different data sets to understand the origins of the genetic diversity in the country. These data sets are called **DS<n>** and are described in **S2 Table**.

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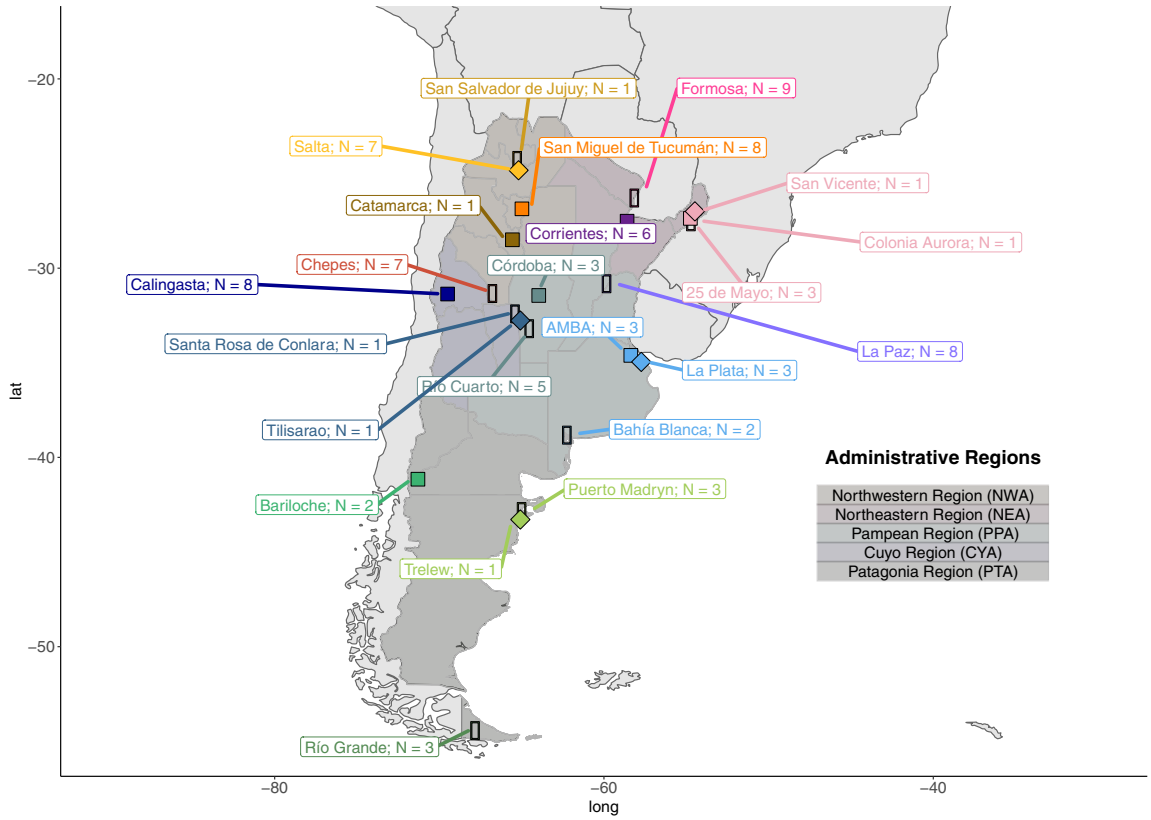

Fig 1. Sample locations from the present study.
The samples are divided according to administrative regions for visualization only.
N: The number of samples that passed genotyping Quality Controls.

4.1 Ancestry in a worldwide context

In order to characterize the genetic diversity observed in Argentina within a worldwide genetic context, Principal Component Analysis (PCA [44]; **S1 Fig**) was applied to the dataset **DS1,** which contains genotype data for the samples from Argentina generated in the present study, as well as admixed and Native American individuals from South America characterized in previous studies [33,37,38] and individuals from Europe and Africa from the 1000 Genomes Project (1KGP; [45]). The PCA shows, as expected, that Argentinean individuals have different proportions of European, Native American and, much less represented, African

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ancestry (**S1A Fig**). This pattern, which has already been documented in other admixed populations in South America [33,45], including in Argentina [14–17,34] supports the heterogeneous genetic origins throughout the country. The third and the fifth principal components discriminate three different main Native American ancestries that are also represented in the Argentinean samples. PC4 discriminates between Southern and Northern European individuals (**S1B Fig**) while PC6 divides Luhya population, a Bantu-speaking population in Kenya, to Western African populations (**S1C Fig**). The Argentinean samples do not exhibit any striking gradient along these two PCs. The ancestry composition of the Argentinean individuals will be discussed below.

We ran unsupervised clustering models with Admixture software [46] on **DS1**. We used from 3 to 12 putative ancestral populations (*K* ranging from 3 to 12). Comparing the cross-validation scores obtained for each run, we estimated that the genotype data analyzed was best explained with a model with *K*=8 ancestral populations (**S2A Fig**). At *K*=3 (**S2B Fig**), the algorithm allows estimating the proportions of European, Native American and African ancestry. For *K*=4 to *K*=7, (**S2C-F Figs**), the model detects sub-continental ancestries, i.e. it divides the main continental ancestry into different components, that are finally all observed in the model with *K=8* (**S2G Fig**). That is, the European ancestry is divided into Northern and Southern components, while African ancestry is composed of Westernmost African, Gulf of Guinea and Bantu-influenced components. Moreover, three components are observed for Native American ancestry: one represented in Central Chile/Patagonia populations (hereafter referred to as **CCP**), another present in

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Central Andes populations (hereafter referred to as **CAN**) and a third one in populations mainly from Lowlands (here after referred to as **LWL**).

S3 Table lists, for individuals included in **DS1**, the proportion of continental ancestry estimated from the model with *K*=3, its proportion of sub-continental component estimated from the model with *K*=8, as well as the proportion of continental ancestry estimated from *K*=8 (obtained from the sum of the sub-continental component proportions). We confirmed that the estimates of the continental ancestry proportion obtained from a model with *K*=3 and *K*=8 are highly consistent (**S3 Fig**).

From the Admixture results for *K*=8 (**S2G Fig**), we observed that Peruvian and Chilean admixed samples [33] exhibit very low northern European ancestry proportions. The Native American ancestry for Peruvian admixed samples is mainly represented by CAN, although the Lowlands component is present in smaller proportions. Moreover, the Native American ancestry for the Chilean samples is mainly characterized by CCP. As for the Argentinean samples from both the present study and from [33], their European ancestry is divided by Southern and Northern components, the former being the most abundant. Arguably surprisingly, all three components of Native American ancestry are present in most Argentinean samples (**S2G Fig**). The low proportions of African ancestry in Argentinean samples makes difficult to interpret its sub-continental origins from analyses within a global context.

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The Native American ancestry proportions are higher in our samples than in the Argentinean sample studied by [33] (**S4C Fig**; Wilcoxon test $P = 2.57x10^{-8}$). This could demonstrate that the selection of individuals with previously characterized uniparental Native American lineages can improve the overall representation of Native American ancestry in autosomal genomic regions, which would refute a previous study in Argentina [12]. However, this hypothesis can not be formally proven since our sample is almost exclusively composed of individuals with Native American uniparental lineages, and the genotyping data from [33] does not allow the inference of the uniparental lineages. Alternatively, this significant difference could also underscore that the exclusively urban samples from [33] do not allow the study of the total extent of Native American ancestry in the country. Moreover, the proportions of African ancestry are slightly higher in our samples than in the Argentinean sample studied by [33] (**S4A Fig** Wilcoxon test; $P = 2.40x10^{-2}$). Altogether, our sample is suitable for further studying the origins of Native American and African ancestry in Argentina.

4.2 Sub-continental ancestry components in Argentina

4.2.1 Local Ancestry Estimates

To explore the origins of the three main continental ancestries at a finer geographical level, we first estimated local ancestry patterns in phased **DS2** and **DS3** (**DS2p** and **DS3p**) separately. Across phased chromosomes, we assigned whether a genomic region was of Native American, European or African ancestry using RFMix v2 [47]. A sanity check confirmed that the ancestry proportions across

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the genome estimated by local and global ancestry approaches are concordant (**S5 Fig**). It seems that local ancestry, as compared to global ancestry, tends to overestimate the proportion of the genome with European ancestry while underestimating the proportion of African and Native American ancestry. The present article main conclusions concern the origins of African and Native American ancestries, and we argue that our approach of assigning regions to one of those two ancestries is conservative.

Next, we masked the data in order to keep, for each individual, only the regions with ditypes of a given ancestry, i.e. regions that exhibit the ancestry of interest on both chromosomes (**S6 Fig**; for details see Material and Methods).

To decipher the sub-continental origin(s) of the European, African and Native American ancestries segregating in Argentina, we applied principal component and unsupervised clustering analyses on the masked data compiled with reference data sets describing the genetic diversity within each continent (**S2 Table**).

4.2.2 European ancestry components in Argentina

To explore the European origins of the Argentinean, we used **DS4**, a combination of the masked genotype data for admixed individuals with a set of individuals carefully selected to be representative of the genetic diversity in their sampling area [48,49]. We estimated that the genotype data analyzed was best explained with a model with K=2 ancestral populations for the unsupervised clustering model (**S7A Fig**). Although it is rather difficult to assign a clear label to each of these two ancestral populations, it seems that the algorithm discriminates between Northern and

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Southern Europe components (**S7B Fig**). For *K*=3, we observed that Iberians individuals exhibited a higher proportion of a new component highly represented in Southern American samples (**S7C Fig**). This pattern most likely reflects the legacy of first Iberian migrations in South America during colonial period [12].

From the PCA applied on the same dataset (**DS4)**, we observed, as previously described [33,34], that most of the European ancestry of admixed Argentinean individuals clusters with Iberians and Italians (**S8 Fig**). However, some individuals trace their European ancestry back to Central or Northern Europe. Both PCA and Admixture showed that the individuals with higher proportion of Central / Northern Europe ancestry are from Misiones province (Northeastern region) (**S7** and **S8 Figs**), consistent with the historical record of settlement of Polish, German, Danish and Swedish colonies promoted by governmental or private enterprises in the province [50]. This demonstrates that to identify the genetic legacy from secondary migration streams and thus better reflect the evolutionary history of populations in Argentina, it is primordial to increase the number of studied individuals in the country.

4.2.3 African ancestry components in Argentina

To investigate the sub-continental components that explains African ancestry in Argentina, we used **DS5** which combines masked genotype data from admixed individuals with a published data set of African individuals [45,51–53] (**Fig 2A**). The African reference populations used here can be divided into five main groups:

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Bantu-influenced, Hunter-Gatherers, Western Africa, San and Eastern Africa populations [51].

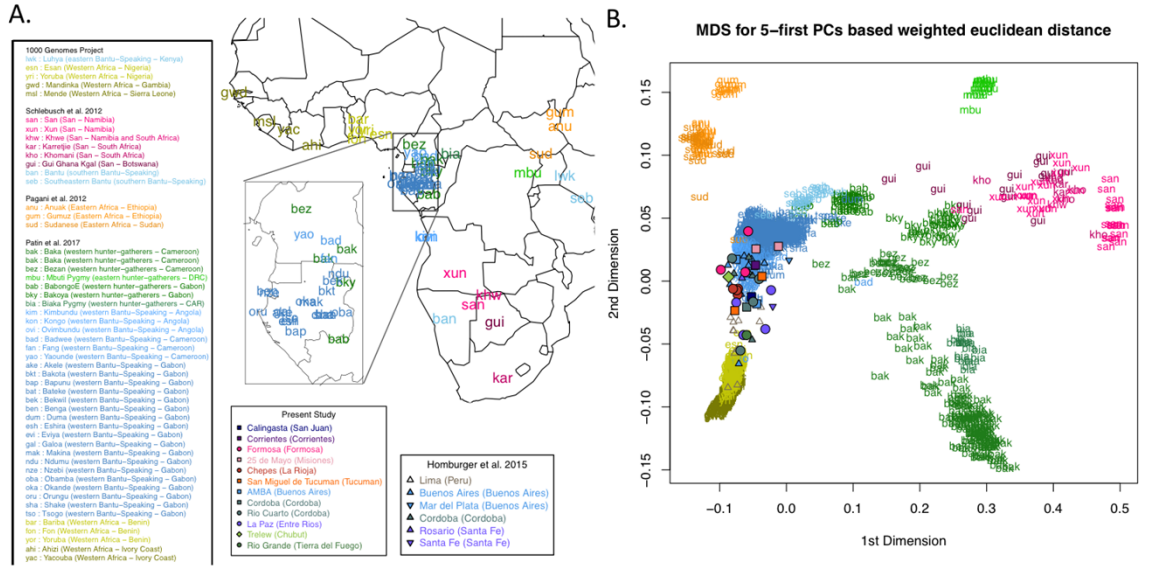

Fig 2. African Ancestry-Specific Principal Component Analysis. **(A)** Localization map of the African reference samples. **(B)** Multidimensional scaling scatterplot (MDS) from Euclidian distances calculated from weighted 5-first African-specific Principal Components. Elbow method to choose the best number of PCs to compute MDS is shown in **S9 Fig.**

The PCA applied to this data allowed the distinction among those groups (**Fig 2B**). The individuals from Lima (Peru) tend to group with the Western Africa populations, consistent with historic records that account to a Western African origin of the slaves deported to Peru through the Caribbean harbors [54]. Most of the Argentinean individuals are within the Bantu-influenced population cluster while others individuals are clustering with Western Africa populations. Several other Argentinean individuals do not group within any African cluster and rather are found between the Western Africa and Bantu-influenced clusters (**Fig 2B**). These patterns were confirmed by applying Admixture algorithm. The Cross-

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Validation procedure points to a best fit of the data with *K*=5 (**S10A Fig**). With five ancestral populations, African individuals cluster in a similar fashion as observed in the PCA. The Bantu-influenced and Western Africa components are the most represented in Argentinean individuals. Moreover, this analysis also showed that some individuals exhibit smaller proportions of Eastern Africa component, particularly in Northern Argentina, as well as Western Hunter-Gatherer ancestry (**Fig 3**).

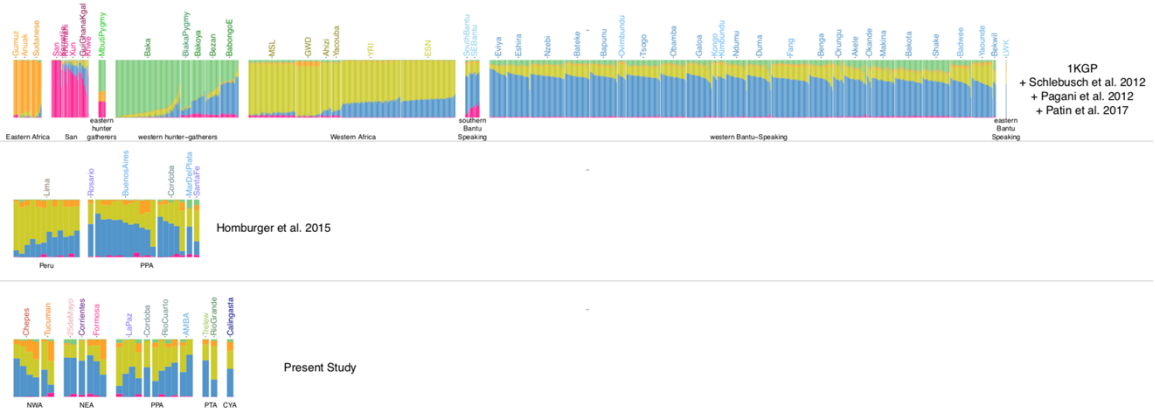

Fig 3. African Ancestry-Specific Admixture Analysis.
Admixture plots for *K=5*

The legacy of Western Africa on the African genetic diversity in the Americas has been preeminent [55–57], along with an impact of Bantu-influenced populations from Central Western Africa, particularly in Brazil South and the Caribbean [55,56]. These two African ancestries have also been previously documented in Argentina from studies of autosomal [34] and maternal markers [58,59]. Maternal lineages specific to populations from Central Western Africa – particularly from Angola - are the most common African lineages in Argentina according to studies in the Central region [58] and in four urban centers (Puerto Madryn, Rosario, Resistencia and

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Salta) [59]. These results are concordant with the predominance of the Bantu-influenced origin identified in the present study from autosomal markers. In addition, the presence of Southeastern Africa maternal lineages in Argentina [58,59] is consistent with African ancestry of this origin identified here. Additional analyses with larger number of individuals and higher SNP density for autosomal are required to follow-up these results.

4.2.4 Native American ancestry components in Argentina

We compared masked genotype data for admixed individuals with masked genotype data of Native American reference populations from South America [37,38] and a set of ancient DNA data for samples in the region [35–37,60,61] (**DS6**; **Fig 4A**). The PCA using modern individuals, and projecting the ancient samples, confirmed the three main South Native American clusters described by de la Fuente *et al.* (2018) (**Fig 4B**). Namely, we can distinguish between the three clusters already mentioned in the analyses at the global level: CAN, CCP and LWL. The admixed individuals from Lima are mainly located within CAN, while those from Santiago de Chile are found next to CCP. As for the Argentinean admixed individuals, some are located within one of the three clusters while many others are located in-between.

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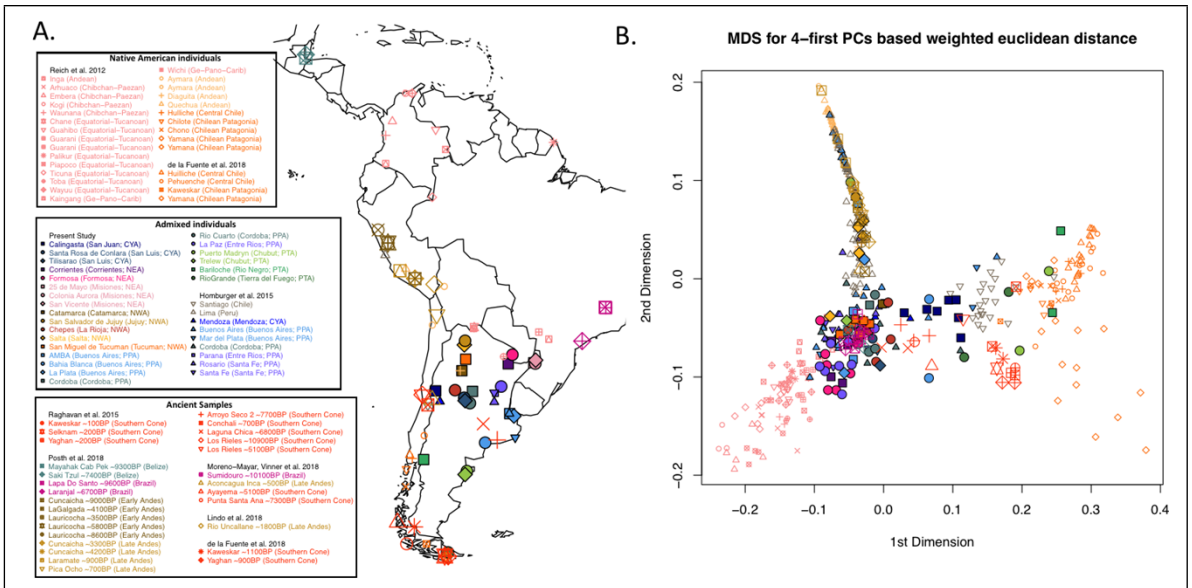

Fig 4. Native American Ancestry-Specific Principal Component Analysis. **(A)** Localization map of the samples. **(B)** Multidimensional scaling scatterplot from Euclidian distance calculated from weighted Native American ancestry specific Principal Components. Elbow method to choose the best number of PCs to compute MDS is shown in **S11 Fig.**

Similar results were also observed when applying Admixture algorithm. The Cross-Validation procedure points to a best fit of the data with *K*=3 (**S12A Fig**). The proportion estimates for the three main Native American ancestries observed for the South America Native American individuals and for the ancient samples are consistent with CAN, CCP and LWL labels that we attributed for the interpretation of the PCA (**Fig 5**). Concerning the admixed individuals, CCP ancestry is the most represented in Santiago de Chile, while CAN ancestry prevails in Lima, Peru (**Fig 5**). In Argentina, the three Native American ancestries are observed in almost all the admixed individuals studied. In Northeastern Argentina (NEA), LWL ancestry is the most frequent. In Northwestern Argentina (NWA), CAN ancestry is not clearly dominating since LWL is also observed in important proportions. In the South, CCP is observed in greater proportions. However, two individuals sampled in Trelew and

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Puerto Madryn, next to the Atlantic coast of Patagonia, exhibit greater proportions of LWL and CAN, respectively (**Fig 5**). The reduced genealogical information for the individual from Trelew does not inform about his Native American ancestors. On the other hand genealogical information of the individual from Puerto Madryn attests to a recent migration to this locality since he was born in Jujuy province (NWA), as well as her both parents and paternal grandparents while maternal grandparents were born in Bolivia. This particular case is a perfect example of recent migrations to the Patagonia region [15], and more generally within or to Argentina [62].

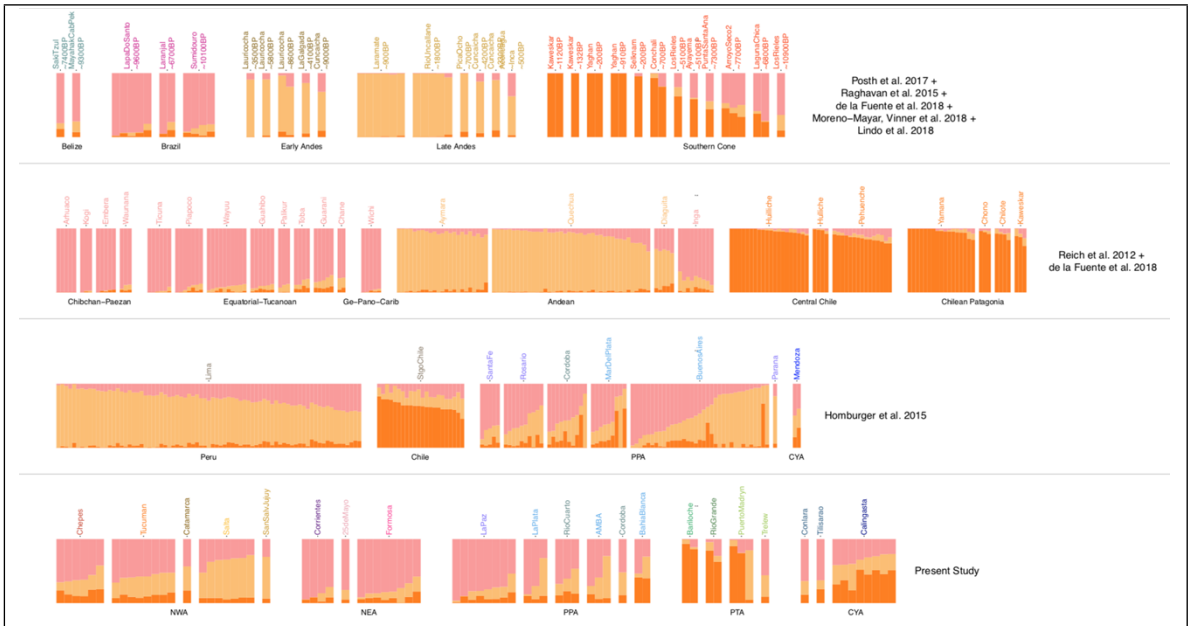

Fig 5. Native American Ancestry-Specific Admixture Analysis.
Admixture plots for *K=3*

Although migration events tend to reduce the genetic distance among these components, we still identified correlations between geographical coordinates and the proportions of each Native American ancestry component. Indeed, the proportion of CAN and LWL is higher further North (**S13A-B Fig**) while the

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proportion of CCP is higher further South (**S13C Fig**). In addition, the proportion of CAN is higher further West (**S13D Fig**) while the proportion of LWL is higher further East (**S13E Fig**). This association between Native American ancestry proportions and geography is expected under a model of ancient divergence and genetic drift.

Note that a model with *K=3* at the South American scale with masked data is consistent with the results from Admixture at a global scale with unmasked data for which the best model contains 3 putative ancestral populations specific to South American (**S2 Fig**). Moreover, the proportions for each of the three Native American components estimated from masked data highly correlate with estimates from unmasked data (**S14 Fig**).

Many individuals from the Cuyo and Pampean regions of Argentina (San Juan and Córdoba provinces as well as Southern of Buenos Aires province) exhibit a complex Native American ancestry in both the Principal Component (**Fig 4**) and Admixture analyses (**Fig 5**). This pattern of intermediate position in the PCA graph and with mid proportions of different ancestries in Admixture analyses can be interpreted as the result of a mixture between the ancestries depicted. Alternatively, it can reflect genetic diversity underrepresented by these ancestries because of relative limited shared history with them. Increasing the number of ancestral populations to 4 in the Admixture analyses, we observed that CCP component is actually structured and that modern individuals from Argentinean Patagonia exhibit mainly a Central Chile ancestry instead of a Patagonian ancestry (*K*=4; **S12C Fig**). With an additional

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ancestral population in the model, we identified a component specific to the Middle Holocene samples from the Southern Cone and Brazil (*K*=5; **S12D Fig**).

In order to analyze further the evolutionary history of the Native American ancestry, we assigned the 452 modern South American individuals (the 53 ancient samples included in **DS6** were not analyzed here) to a Native American ancestry cluster using an objective quantitative approach based on *K*-means clustering (**S15 Fig**; see Material and Methods for details). Thus, we assigned 163, 161 and 70 individuals to the clusters representing CAN, LWL and CCP, respectively. 32 individuals were assigned to a fourth cluster. The remaining 26 individuals were removed for further analyses because their group assignation was not consistent across the three clustering approaches. **S4 Table** describes the cluster assignation for the studied individuals. We acknowledge that these groups are culturally, ethnically and linguistically heterogeneous. However, we argue that analyzing such groups built from genetic similarities may provide interesting insights at a broad scale into evolutionary mechanisms that shaped the Native American ancestry in South America.

4.2.5 Relationship among the four identified Native American components

We ran f_3*(Target; S1, S2*) to test whether *Target*, *S1* and *S2* are related in a form of a tree (positive f_3 expected) or whether *Target* is the result of admixture between *S1* and *S2* (negative f_3 expected). Significant f_3 positive scores were obtained for all the possible comparisons among the four Native American clusters identified (**Fig 6A**; **S5A Table**). In addition to confirming the differentiation among the three

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components labeled geographically, this analysis showed that the fourth group most likely represents a Native American component on its own never described in any previous study of autosomal genetic markers. Actually, the distribution of 1 –*f3(YRI; Ind1, Ind2)* between pairs of individuals from different groups (**S16 Fig**) does not suggest a mixture among the 3 geographically labeled components to explain why individuals from the fourth group are located at intermediate positions in the PCA and exhibit mid Admixture proportion estimates (as shown in **Fig 4** and **Fig 5**).

We estimated $f_4(YRI, Target; S1, S2)$ to test whether a component (*Target*) shares more affinity with any of the other two components (*S1* and *S2*). We observed that (i) CAN has no particular genetic affinity with any component relative to the others; (ii) LWL is closer to CAN as compared to CCP and the fourth component; and (iii) the fourth component and CCP exhibit higher genetic affinity between them than with CAN or LWL (**Fig 6B**; **S5B Table**). However, a neighbor-joining analysis [63] from distances of the form $1/f_3(YRI; X, Y)$ suggests that CAN is more closely related to CCP and the fourth component than to LWL (**Fig 6C**; **S5C Table**).

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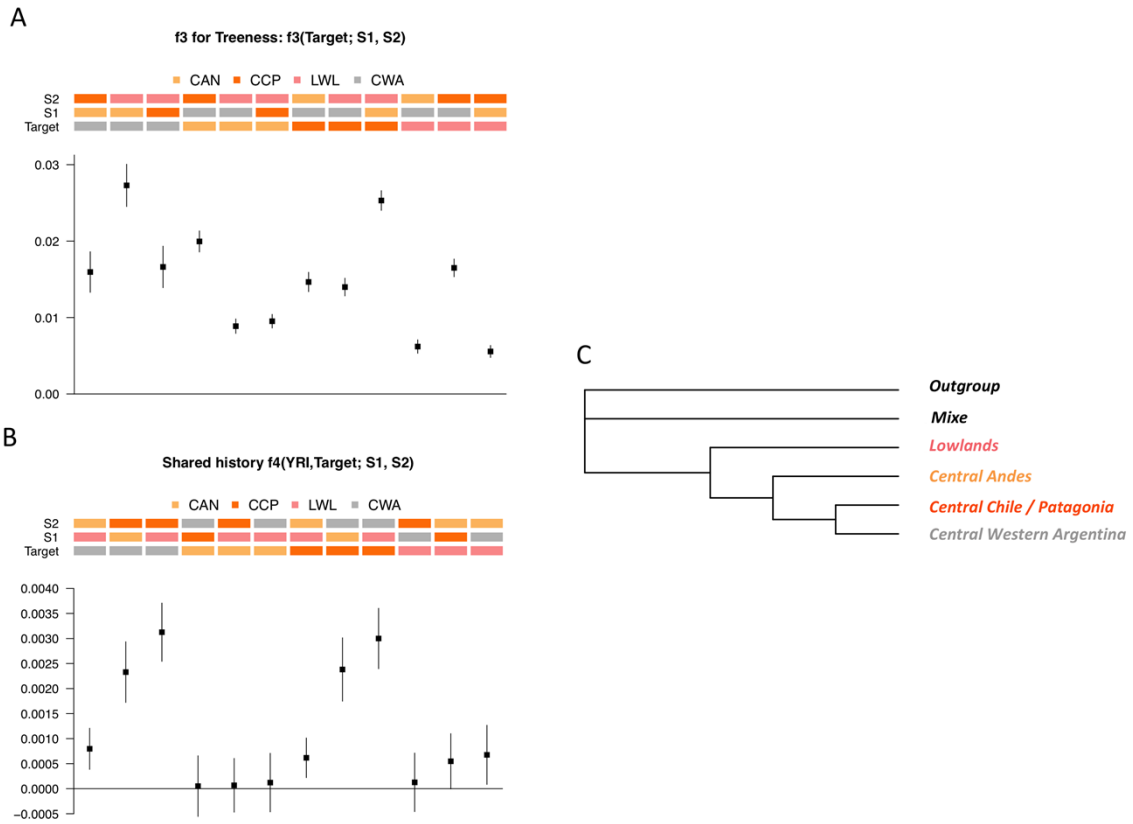

Fig 6. Relationship among the four Native American groups identified. **(A)** f_3(*Target; S1, S2*) to test for treeness. **(B)** f_4*(YRI, Target; S1, S2)* to test whether Target shares more ancestry with S1 or S2. Since f_4 is symmetrical switching *S1* and *S2,* only positive comparisons are shown. Vertical segments are the +/- 3 standard errors intervals in A and B. **(C)** Neighbor-joining tree estimated from distance matrix. Distances were estimated from 1/ f_3(YRI; X, Y). The tree was estimated using ancient sample from Upward Sun River site in Beringia (USR1) as outgroup CAN: Central Andes; LWL: Lowlands; CCP: Central Chile / Patagonia; CWA: Central Western Argentina; YRI: Yoruba from 1KGP.

Seven out of eight individuals from Calingasta, a locality in the San Juan province and located in the Northwest Monte and Thistle of the Prepuna ecoregion, were assigned to the fourth cluster (the only remaining individual from this locality is not consistently assigned to a cluster and has been removed). Moreover, out of 18 individuals from Santiago de Chile assigned to any given cluster, 16 have also been assigned to the fourth component. The genealogical record for the studied

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Calingasta individuals attests to a local origin of their direct ancestors up to two generations ago and their region-specific maternal lineages (**S1 Table**; [64]). Moreover, all the Huilliche and Pehuenche individuals from Central Chile [37] have been consistently assigned to CCP. Altogether, we argue that this fourth component may represent a Native American ancestry that diverged from CCP and established on the Eastern side of the Andes in Cuyo region. It may have extended its influence to the Pampas and Argentinean Patagonia region as suggested by the assignation to this cluster of individuals from the provinces of Mendoza, Córdoba Capital, Buenos Aires and Tierra del Fuego (**S15 Fig**; **S4 Table**).

The existence of a specific differentiated component in the Central Western and Central regions of Argentina has been previously suggested from mitochondrial analyses accounting to the genetic relationship between these two regions [11] and the presence of specific clades underrepresented elsewhere [11,58,65]. These studies support the hypothesis of a common origin and/or important gene flow [66], and the authors referred to a meta-population with great temporal depth and differentiated from other regions in Argentina. Moreover, the ethnographic description of the populations that were settled at the moment of contact with the Spanish colonies accounts to a potential relationship between Huarpes in the Cuyo region and Comechingones in present-day Córdoba province ([67], cited by [68]). Thus, we suggest labeling as Central Western Argentina (**CWA**) this fourth Native American component never described before from autosomal markers.

Moreover, different, perhaps complementary, historical facts may explain the representation of this component in Santiago de Chile and its absence in Huilliche

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and Pehuenche populations. First, in pre-Colombian times, Central Andean civilizations may never have reached Southern latitudes than the Biobío River. Indeed, the troops of the Inca Empire, the Central Andean civilization that reached the highest latitudes, apparently never crossed that river [69,70], leaving Huilliche and Pehuenche territories, among others, outreach the expansion of any Central Andean civilization [69]. Second, during colonial times, the Spanish colonies could not settle South of the Biobío River neither, and they organized massive deportation of Huarpes individuals from Cuyo region to palliate the lack of indigenous workforce [71]. For example, in Santiago de Chile, in 1614, 37% of the indigenous people that lived in the suburbs were Huarpes according to the chronicler Vázquez de Espinosa [72].

The present study, in which we analyzed individuals that do not belong to any indigenous community, made possible the identification of a Native American component never described previously from autosomal markers. This demonstrates the importance of broadening studies beyond the indigenous communities in order to reduce the underrepresentation of many regions in Argentina, and thus, to reach a better characterization of the Native American specific genetic diversity.

4.2.6 Genetic affinity of the four Native American ancestry components with ancient populations

Pseudo-haploid genotype data were used for ancient samples published in the literature [35–37,60,61,73,74], and their genetic affinity to the four Native American ancestry components identified in Argentina was evaluated. The ancient samples

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were classified according to their spatiotemporal distribution following Posth *et al.* 2018 [35].

Graphical summaries of pairwise distances are available as the neighbor-joining analysis [63] from $1/f_3(YRI; X, Y)$ distances (**S17A Fig**) and a Multidimensional scaling analyses from $1-f_3(YRI; X, Y)$ distances (**S17B Fig**).

When comparing the genetic affinity of a given component with the different ancient groups using either the *f3-outgroup* or the *f4* statistics (**S18 Fig; S5D,E Tables**), we identified that CAN tends to exhibit greater genetic affinity with ancient Andean populations than with other ancient groups (**S18A,E Figs**). Strikingly, the genetic affinity of this component with both Late Andes and Early Andes ancient groups would point to a genetic continuity across the whole temporal transect that the archaeological samples provide. This would suppose that the replacement of an early population arrival by a later stream of gene flow in Central Andes identified previously [35] does not fully explain the current-day genetic diversity for CAN. For CCP, we observed higher genetic affinity with ancient Southern Cone groups (**S18C,G Figs**). Overall CWA appears to be closer to ancient Southern Cone groups, particularly with Conchalí ~ 700BP, although its genetic affinity with some Andean groups (La Galgada ~4100 BP, Pica Ocho ~700 BP and Aconcagua ~500 BP) is relatively important (**S18D,H Figs**). As for LWL, we observed intermediate genetic affinity with ancient samples from both the Andes and the Southern Cone (**S18B,F Figs**). Indeed, the three ancient samples with the greatest genetic affinity with Lowlands components are from the Andes (La Galgada ~4100 BP, Aconcagua ~500 BP, and Pica Ocho ~700 BP), followed by three ancient groups in the Southern Cone

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(Conchalí ~700 BP, Yaghan ~ 200 BP, and Selk'nam ~200 BP). The fact that there is no ancient sample group exhibiting outstanding genetic affinity with LWL points to the underrepresentation of this component in ancient samples. Indeed, the temporal period covered by the Brazilian ancient samples is much older than for other regions, and the geographical range restricted to Brazil does not cover the entire area where LWL currently prevails. Moreover, LWL is a heterogeneous group that includes individuals from the Gran Chaco in Argentina, the Amazonas and Northern Andes. Even so, Admixture analyses demonstrated that LWL shares more ancestry with ancient samples in Brazil (**Fig 5**).

To study further the history of the four modern components of Native American ancestry, we evaluated the relationship of the time depth of the ancient samples from either the Andes or the Southern Cone, with their genetic affinity to the modern components of Native American ancestry (**Fig 7**). For that purpose, we performed a linear regression between *f3(*YRI*, X, Anc)* – where *X* is one the four Native American component – and the age of the ancient population tested (*Ancient* in the f_3 formula*).* We observed a significant relationship between the age of the ancient Southern Cone samples and their genetic affinity with CCP ($P = 3.15 \times 10^{-4}$) and CWA ($P = 3.94 \times 10^{-2}$) while no significant relationship was identified for LWL and CAN ($P = 0.523$ and $P=0.596$, respectively; **Fig 7A**). These patterns could be due to a relationship between geography and the age of the ancient samples since the most recent samples are concentrated in the Southern tip of the subcontinent (**Fig 4A**). To correct this putative confounding effect, we repeated the same analyses but using the residuals of the linear regression between the age of the ancient samples

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and their geographic coordinates. This correction intensified the relationship described for CCP and CWA (P = 4.78x10^{-5} and P = 2.21x10^{-6}, respectively; **Fig 7C**). It also allowed to actually identifying significant relationships for LWL and CAN (P = 8.07x10^{-4} and P = 1.56x10^{-3}, respectively). On the other hand, CAN is the only modern Native American component that exhibits a significant relationship between its genetic affinity with ancient Andean samples and their age (**Fig 7B**). This pattern holds the correction for geography (**Fig 7D**). Repeating the same analyses using f_4 statistics, instead of f_3-Outgroup, we reached the same conclusions regarding the relationships between the age of the ancient Andes samples or the Southern Cone ancient samples and their genetic affinity with any of the four modern Native American components (**S19 Fig**).

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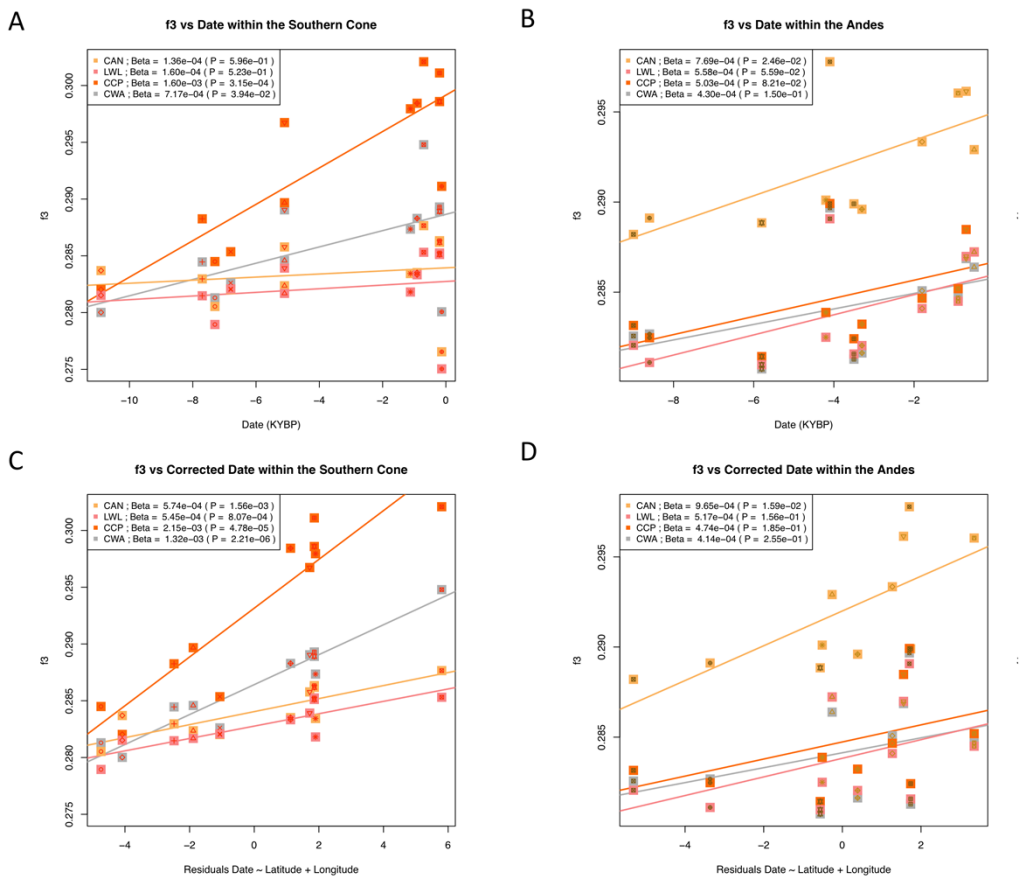

Fig 7. Changes across time of genetic affinity of the four Native American with ancient samples.
Each point represents a f_3-Outgroup score of the form *f3(YRI; X, Ancient)* vs the age of ancient sample, where *X* is one of the four identified modern Native American components, and *Ancient* is an ancient group. *X* is represented by the color of the square while the point within the square represents *Ancient*. The point code of the ancient samples is as shown in **Fig 4. (A)** f_3 vs age of ancient samples from Southern Cone. **(B)** f_3 vs age of ancient samples from Andes. **(C)** f_3 vs age of ancient samples from Southern Cone considering age correction. **(D)** f_3 vs age of ancient samples from Andes considering age correction. Linear regression slopes and the associated *P*-values are shown. CAN: Central Andes; LWL: Lowlands; CCP: Central Chile / Patagonia; CWA: Central Western Argentina; YRI: Yoruba from 1KGP

4.2.7 Comparison of the shared history of two components with ancient DNA samples

The analysis of archaeological samples gives insights into the temporal depth of the main demographic events that occurred during the evolutionary history of the

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analyzed populations. Concerning the times of divergence of the four components discussed in this study, although it is not possible to estimate them exactly, we can establish lower and upper bounds. To explore this issue, we used another setting of the *f4* statistics (**S5F Table**; **S20 Fig**). In this analysis, of the form *f4(YRI, Ancient; X, Y),* a negative result is expected if *X* shares more history with an *Ancient* relative to *Y,* while a positive result is expected if *Y* shares history with *Ancient* relative to *X.* We observed that all Southern Cone ancient samples – except Los Rieles ~10900 BP which has been described as derived from an ancestry shared with the Clovis culture that has been replaced later [35]) – are more similar to CCP than to CWA (**S20F Fig**), pointing to divergence time between those two components older than ~7700 BP. CWA is not more attracted by any Brazilian and Andean ancient sample as compared to CCP, reinforcing the idea that CWA is not a mix of CCP, CAN and LWL related ancestries. Moreover, we observed that all the ancient groups of the Southern Cone (with the exception of Los Rieles ~10900 BP) have greater genetic affinity with CCP than with CAN and LWL (**S20B,D Figs**). With the exceptions of the two Middle Holocene ancient samples from Patagonia (Ayayema ~5100 BP and Punta Santa Ana ~ 7300BP) and Los Rieles ~10900 BP, ancient groups of the Southern Cone exhibit higher genetic affinity with CWA than with LWL (**S20C Fig**). In addition, the comparisons including LWL and CWA also point to a closer genetic relation between ancient groups from the Southern Cone with CWA, although significance is reached only for Late Holocene Patagonia samples.

All the ancient samples from the Andes, indifferently if there have been previously assigned to the Early or Late population stream [35], are genetically more similar to

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CAN than to any of the three other components (**S20A,D,E Figs**). These results support the hypothesis that the ancestry associated with an early Andes population arrival remains present to some extent in modern Central Andes populations and has not been totally replaced by a later stream of gene flow. Although, ancient samples from Brazil does not exhibit striking differences in their genetic affinity with a modern Native American component as compared to another, it seems that they are closer to LWL than to CWA and CCP but not than to CAN (**S20A-C Figs**). Moreover, the archaeological samples in Brazil have higher genetic affinity with CAN than with CWA and CCP (not significant for the latter comparisons). This points that divergence of LWL with CCP and CWA could have occurred before than the divergence of LWL with CAN (also observed in **S17A Fig**).

Altogether, the trends depicted by these analyzes are expected under a model of early divergence among the four components. Our results also advocate to genetic continuity over long period of time for the different components after they differentiated.

4.2.8 Past influence of a component on another since their divergence

In order to get insights into the past genetic influence between two components since their divergence, we applied a last *f4*-statistics analysis (**S5G Table**; **S21 Fig**). In this analysis of the form *f4(Ancient, X; Y, YRI),* a negative result is expected if *Y* shares history with *X* relative to *Ancient,* while a positive result is expected if *Y* shares history with *Ancient* relative to *X.* We observed that the influence of LWL into the three other components was relatively limited (**S21A,C,E Figs**). However, this

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result suffers poor resolution due the temporal and geographical gaps in the ancient samples representing this component. The influence of CAN appears to have been more pronounced into LWL component than into CWA and CCP components (**S21B,G,I Figs**). Strikingly, Pica Ocho ~700 BP, La Galgada ~4100 BP and Aconcagua ~500 BP attract any of the four components relative to any other component (not always reaching |Z| >3), with the exception of comparisons including simultaneously CCP and CWA components. In other words, the relations of a given modern component with Pica Ocho ~700 BP, La Galgada ~4100 BP and Aconcagua ~500 BP are stronger than with any other modern component, except when including both CWA and CCP in the comparison. The genetic influence of these three Andes ancient groups outside the Central Andes may be related to events geographical expansions of some Andean populations at given times. Discussing this pattern further is beyond the scope of the present study.

The influence of CCP into LWL and CAN also appears to have been reduced, although the negative *f4* with Kaweskar ~100 BP suggests gene flow between CCP and these two components in very recent times (**S21D,H Figs**).

The mutual influence of CCP and CWA components seems to have been important in remote times (**S21K,L Figs**). However, the available ancient samples provide much more precise information on the influence of CCP into CWA component than for the other way around. Indeed, CWA is not attracted by any ancient DNA group relative to CCP (**S21K Fig**). Although Conchalí ~700 BP exhibit higher genetic affinity with CWA among all the analyzed ancient groups (**S18D,H Figs**), it clearly attracts CCP relative to CWA (**S21L Fig**) while it does not attract CWA relative to CCP. The

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comparisons including CCP and CWA demonstrate that the archaeological record for which genetic data has been generated does not include ancient samples representing CWA since its early divergence with CCP neither ancient samples related to the common ancestors specific to these two components.

4.3 Conclusions

We studied genetic ancestry at the sub-continental scale in Argentinean populations. First, we confirmed that European ancestry is mostly explained by Southern European origins, although we identified several individuals with Northern European ancestry. Second, we described three main components of African ancestry in Argentina. Although the Bantu-influenced and Western components are clearly the most represented, we also found that an Eastern origin can explain some of African ancestry in Argentina and represents up to ~30% of the African ancestry for some Argentinean individuals. Studying more admixed individuals in Argentina would help to get a better representation of the complex origins of African and European ancestries in the country.

Concerning Native American ancestry, we concluded that Argentinian populations share, in varying proportions, at least three distinct components: from the Central Andes, from Central Chile/Patagonia and from the Lowlands. These components correlate with geographic coordinates. Moreover, we present here some arguments that the Native American ancestry in the Central Western region of Argentina may actually derive from a fourth component that diverged early from the other Native American components, and it maintained a tight link with the Central

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Chile/Patagonia component. This relation is not explained by a putative contribution of admixed individuals from Santiago de Chile (**S22 Fig**). Having identified this component from admixed individuals demonstrates that to characterize the Native American genetic diversity focusing only on indigenous communities is insufficient, at least in Argentina.

It is important to acknowledge that our results should be taken cautiously since studying admixed individuals make the analyses more complex. Leveraging a pure statistical approach, we grouped individuals from rather culturally, ethnically and linguistically heterogeneous groups to represent the four Native American components discussed here. These groups exhibit within population structure, and gene flow are most likely to have occurred among them after divergence. In addition, temporal and geographical gaps in the archaeological samples analyzed, particularly for the Central Western Argentina and Lowlands components, limits the interpretative resolution of our analyses. Yet, the present study provides useful insights into the routes followed by the main populations arrivals in the Southern Cone (**S23 Fig**).

Further efforts are needed to better characterize the Native American ancestry component identified in the Central Western region of Argentina. Particularly we encourage future studies to confirm the tentative geographical label that we suggest here, and to estimate its influence in the region. Besides these specific questions, many other general questions remain to be answered to better understand the pre-Colombian population dynamics in the Southern Cone such as the time and place of the splits among the components described here, and the extent of genetic

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exchanges among them. More genotype data for ancient samples, modern indigenous communities and admixed individuals, particularly in Central, Northeastern and Patagonia regions of Argentina, would help to decipher these issues.

The genomic characterization of populations is an unavoidable practice for many issues ranging from the understanding of our biological heritage, the rational use of biobanks, the definition of an adequate reference genome, the estimation of polygenic risk scores, the study and treatment of simple and complex diseases, and the design of a national program of genomic medicine in our country. This study is a common effort of exclusively Argentinean laboratories and totally financed by the national scientific system. It is the first step of the *Consorcio Poblar*, a national consortium for the creation of a public reference biobank to support biomedical genomic research in the Republic of Argentina [75]. Genomic knowledge of local populations should be a priority of developing countries for unbiased representation of public databases and the scientific development of our countries.

5 Material and Methods

We genotyped 94 individuals with the Axiom LAT1 array (Affymetrix) from 24 localities and 17 provinces across Argentina (**Fig 1**). These samples were selected among 240 collected by different population genetics groups (*Consorcio PoblAR*) in the country. Besides ensuring an extended geographical range, inclusion criteria were: (i) sufficient DNA concentration; (ii) Native American maternal lineage; (iii)

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Native American paternal lineage (for males when available). All saliva and blood samples have been collected with informed consent for research use, and we dispose of the necessary approvals from different ethical committees in Argentina.

87 samples and 791,543 autosomal variants passed the standard Affymetrix genotyping Quality Controls (**S1 Table**).

Most of the genotype data processing was performed using in-house scripts in R [76] and perl [77], leveraging *plink2* [78], *vcftools v1.13* [79], and *bedTools v2* [80].

We compiled the genotype data for the 87 Argentinean samples with different genotype data available in the literature. We focused our study on biallelic SNPs (removing indels and SNPs with more than 2 alleles). Any putative inconsistent strand had been fixed processing to the relevant flip, filtering out any SNP with ambiguous genotype (A/T, G/C).

Cryptic relatedness among samples were assessed using King software [81]. To avoid any 1st degree relationship, we filtered out individuals, minimizing the total number of removals. No admixed individuals had been removed at this step.

In order to search for the fine-scale genetic structure of Argentinean we used different data through this work. **S2 Table** depicts the main dataset arrangements (named **DS<n>**).

5.1 Argentinean genetic diversity within a worldwide context

To analyze genetic diversity in Argentina within a worldwide context, we built the Dataset1 (**DS1**). This dataset contains 87 Argentinean samples, 654 African, 503

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European and 347 American samples from 1KGP [45], 54 modern unrelated Chilean samples [37], and 161 Native-American individuals from South America [38]. Moreover, **DS1** included genotype data from [33] which consists in 82 individuals from Lima (Peru), 27 from Santiago de Chile, and 161 from Argentinean urban centers.

We filtered out any variant and individual exhibiting in the compiled data set more than 2% and 5% of missing genotypes (--geno 0.02 and --mind 0.05 flags in plink 1.9), respectively, as well as Minor Allele Frequency below 1% (--maf 0.01 flag in plink 1.9). The filtered data was then pruned for Linkage-Disequilibrium (--indep-pairwise 50 5 0.5 option in plink 1.9). The combined data set have a total intersection of 59,237 SNPs and 2,076 individuals. With this curated data we performed Principal Component Analyses [44] and Admixture [46] (**S1 and S2 Figs**).

5.2 Local Ancestry

Local ancestry analyses rely on haplotype reconstruction (phasing) and require high SNP density. Since, admixed individuals of interest were genotyped on different microarray platforms, we decided to perform two separate local ancestry analyses on two different data sets (**DS2** and **DS3**). **DS2** consists of 87 Argentinean individuals from the present study, and 54 Chilean individuals [37], all of them genotyped with the Axiom LAT1 microarray. **DS3** includes 175 Argentinean, 27 Chilean, and 119 Peruvian genotyped with the Illumina OMNI1 microarray [33]. Both **DS2**, **DS3** were merged with 1KGP data consisting in 503 phased reference

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samples for each of the African and European genetic ancestry, and 347 Latin American individuals.

The 87 Argentinean, and the 54 Chilean samples were phased with shapeIT2 [82,83]. The genetic map, and 5,008 haplotypes panel provided by 1KGP were downloaded from http://mathgen.stats.ox.ac.uk/impute/1000GP_Phase3/. Algorithm, and model parameters were used by default, filtering out monomorphic SNPs, and those with more than 2% of missing genotype. We obtained phased genotype data for 608,501 autosomal SNPs. This data was then merged with phased 1KGP genotype data for African, American and European samples described before. Since this data set derived from DS2, we call it **DS2p** (phased DS2).

The 175 Argentinean, 27 Chilean and 119 Peruvian samples from [33] were phased separately using the same procedure with shapeIT2. After filtering for missing genotypes and merging with phased 1KGP genotype data for African, American and European samples we obtained phased data for 694,626 autosomal SNPs. Since this data set derived from DS3, we call it **DS3p** (phased DS3).

In **DS2** and **DS3** we ran Admixture using K parameter minimizing the Cross-Validation score. We used individuals with more than 99% of Native American ancestry as references for local ancestry estimation. For **DS2** we used $K = 7$, and Native American ancestry was defined as the sum of the two American specific components observed (**S24 Fig**). According to this criterion, 48 individuals were assigned as Native American reference: 20 PEL, 2 MXL, 23 Chilean from [37] (1 Huilliche, 9 Kaweskar, and 13 Pehuenche), and 3 Argentinean – 1 from the

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Metropolitan Area of Buenos Aires (AMBA), 1 from San Salvador de Jujuy, and 1 from Salta). All the other American samples were defined as Admixed.

For **DS3**, we used K = 5, and Native American ancestry was defined as the single American specific component observed (**S25 Fig**). According to this criterion, 19 individuals were assigned as Native American reference: 10 PEL, 5 from Lima, 1 from Santiago de Chile, and 3 Argentineans from Buenos Aires. All the other American samples were defined as Admixed.

RFMix v2 ([47] downloaded at https://github.com/slowkoni/rfmix on 15th August 2018) was run on **DS2P** and **DS3P** separately using parameter settings similar to [84]. The reference panels consist of the African and European samples from 1KGP, as well as Native American individuals identified through Admixture procedure described before. We used 1 Expectation-Maximization iteration (-e 1) using the reference panels in this process (--reanalyze-reference). We used CRF spacing size and random forest window size of 0.2 cM (-c 0.2 and -r 0.2). We use a node size of 5 (-n 5). We set the number of generations since admixture to 11 (-G 11) considering the estimates from [33]. The forward-backward output was then interpreted to assign allele ancestry to the one exhibiting major posterior probability, conditioning that it was greater than 0.9. Otherwise, the allele ancestry was assigned to Unknown (UKN). The global ancestry proportions estimated by this RFMix analysis were compared with those obtained with Admixture software. The global ancestry proportion estimates obtained by both procedures matched: spearman's correlation greater than 0.9 in American samples for any of the 3 continental ancestries (**S5**

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Fig).

5.3 Ancestry Specific Population Structure

In order to analyze the ancestry-specific population structure we masked the data, i.e. for each individual, we assigned missing genotype for any position for which at least one of the two alleles was not assigned to the relevant ancestry. In other words, to study ancestry A, we kept for each individual, regions exhibiting ancestry A on both haplotypes (ditypes) as illustrated in **S6 Fig**.

5.3.1 European ancestry specific population structure

To study European ancestry specific population structure, we analyzed together masked data for this ancestry for individuals from **DS2P** and **DS3P** excluding individuals from Chilean Native American communities [37]. This data was merged with a set of reference individuals with European ancestry [49], which is a subset of the POPRES dataset [48]. We call this data set as **DS4**. We removed individuals with less than 30% SNPs with the ancestry ditypes (--mind 0.7 with *plink* 1.9). We also removed SNPs with more than 50% of missing genotypes (--geno 0.5 with *plink* 1.9). Thus, **DS4** contains 147 modern Argentinean individuals (71 from the present study and 76 from [33]), 4 individuals from Santiago de Chile, 1 from Lima. **DS4** encompasses 60,968 SNPs of which 54,073 remained after LD-pruning (--indep-pairwise 50 5 0.5 flag in *plink2*).

Smartpca from Eigensoft v7.2.0 run on **DS4** [44] with the *lsqproject* option ON. We report the PCA results summarized into a 2-dimensional space by applying

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Multidimensional Scaling on weighted Euclidian distance matrix for the first N PCs. We weighted each PC by the proportion of variance it explains. We selected the N most informative PCs according to the Elbow method on the proportion of explained variance.

Admixture [46] run with *K* ranging from 2 to 10 with cross-validation procedure.

5.3.2 African ancestry specific population structure

To study African ancestry specific population structure, we analyzed together masked data for this ancestry for individuals from **DS2P** and **DS3P**. This data was merged with a compilation of reference individuals with African ancestry from [45,51–53]. We removed African individuals with less than 99% of African ancestry when comparing them to the 2504 individuals from 1KGP (with *K*=7 minimizing cross-validation score; results not shown). We thus reduced the African reference to 1685 individuals. We call as **DS5** the data set containing both the masked data for admixed South American individuals and African reference individuals.

We removed SNPs with more than 10% of missing genotypes (--geno 0.1 with *plink* 1.9), and individuals with less than 5% of the ancestry ditypes (--mind 0.95 with *plink* 1.9). Thus, **DS5** contains, 26 modern Argentinean individuals (all from the present study), and 12 individuals from Lima (*9*). **DS5** consisted in 137,136 SNPs, of which 128,086 remained after LD-pruning (--indep-pairwise 50 5 0.5 flag in *plink2*).

PCA and Admixture following as for European ancestry specific population structure analyses (described before).

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5.3.3 Native American ancestry specific population structure

To study Native American ancestry specific population structure, we analyzed together masked data for this ancestry for individuals from **DS2P** and **DS3P**. This data was merged with pseudo-haploid data for ancient samples within South and Central America [35–37,60,61], as well as with masked data for Native American individuals from [38]. The pseudo-haploid data for ancient samples was downloaded from the Reich Laboratory webpage (https://reich.hms.harvard.edu/downloadable-genotypes-present-day-and-ancient-dna-data-compiled-published-papers) the 15th of May 2019. For each sample, we used annotations (geographical coordinates and approximate date) from the metafile provided at the same url.

We call this data set **DS6.** We removed individuals with less than 30% SNPs with the ancestry ditypes (--mind 0.7 with *plink* 1.9). We also removed SNPs with less than 50% individuals with the ancestry ditypes (--geno 0.5 with *plink* 1.9). **DS6** contains 146 modern Argentinean individuals (74 from the present study and 72 from [33]), 22 individuals from Santiago and 77 from Lima, along with 207 Native South American individuals from [37,38] and 53 ancient samples. **DS6** encompasses 47,003 SNPs, of which 39,423 remained after LD-pruning (--indep-pairwise 50 5 0.5 flag in *plink2*).

PCA and Admixture following as for European ancestry specific population structure analyses (described before), with the difference that the *poplistname* option was set

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for *smartpca* in order to estimate the PC using only modern samples and project the ancient samples.

5.3.4 Statistical assignation of modern South American individuals to Native American components

Given a distance matrix among modern individuals from **DS6**, we performed *K-means* clustering with the number of clusters ranging from 2 to 20 and selected the *K-means* output minimizing the Bayesian Information Criterion (BIC). This procedure was applied to three different distance matrix: (i) Weighted Euclidean distance based on the two first PCs from Principal Component Analysis (ii) Euclidean distance based on the ancestry proportions estimated from Admixture (K=3) and (iii) $1 - f_3(Ind1,Ind2,Yoruba)$, where *Ind1* and *Ind2* are two individuals. In the three cases, the *K*-means procedure minimized the BIC when considering 4 clusters, and the clusters tend to correspond to the three Native American Component discussed in this paper (Central Chile/Patagonia, Lowlands, Andes) along with one laying in-between, and which we finally attributed to Central Western Argentina. However, individual assignment to one of these four clusters was not totally consistent according to the distance matrix we used. To obtain a robust assignation, an individual was assigned to a given cluster when it consistently belonged to it across the three *K*-means procedures (**S15 Fig**), otherwise it had been removed for following analyses. The cluster assignation for each individual is given in **S4 Table**.

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5.3.5 *F*-statistics to infer relationship between the four Native American components in Argentina and their genetic affinity with ancient populations

Starting from genotype data for the individuals in **DS6**, we also included genotype data from Yoruba (YRI) population from [45], Mixe population from [38] and, pseudo-haploid data for Anzick individual from the Clovis culture [74] and USR1 individual from Upward Sun River in Beringia [73]. The resulting data set is called **DS7**. Within **DS7,** we grouped modern individuals according to their assigned Native American group and we removed those with inconsistent assignation (**S15 Fig, S4 Table**). Thus, **DS7** contains 426 modern individuals, 55 ancient samples, 108 Yoruba and 17 Mixe individuals. **DS7** encompasses 88,564 SNPs. Note that we did not apply any SNP filtering overall **DS7** in order to maximize the number of SNPs included in each group comparison considered.

Using modern individuals from **DS7**, and considering any possible combination of the four groups identified (LWL, CCP, CAN and CWA), we computed the *f3* statistics [85] in the form of *f3(Target; S1, S2).* This allowed to contrast whether *Target, S1* and *S2* could be organized in the form of a phylogenetic tree *(*positive *f3)* or whether the *Target* group is the result of an admixture event between *S1* and *S2* groups (negative *f3*). We also computed *f4(YRI, Target; S1, S2)* to test whether group *Target* shares more evolutionary history with group *S1* (negative *f4)* or group *S2* (positive *f4*). Moreover, we reconstructed the Neighbour-joining tree from the matrix of distances with Phylip v3.2 [63] and *USR1* ancient sample from the Upward Sun

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River Site in Beringia [73] as outgroup. The distances were estimated as 1/ *f3-outgroup(YRI; X, Y).*

We computed f_3-Outgroup and f_4 statistics in order to estimate the genetic affinity of the four Native American components with ancient populations from South and Central America. We computed the *f3-outgroup* and *f4* statistics in the form of *f3(Yoruba; X, Ancient)* and *f4(Yoruba, X; Ancient Beringia, Ancient)*, where *X* the represent the cluster containing all individuals assigned to a given Native American ancestry component, Ancient Beringia is the pseudo-haploid individual USR1 from the Upward Sun River site in Alaska [73].

We also computed f_4 of the form *f4(YRI, Ancient; X, Y)*. This *f4* setting allowed testing which of *X* or *Y,* each referring to one of the four Native American components. shares more ancestry with a given *Ancient* group. *X* and *Y*.

We finally computed f_4 of the form *f4(Ancient, X; Y, YRI)* to test whether a given modern Native American component *Y* shares exhibit closer genetic affinity with a given *Ancient* group (negative f_4) or with another modern Native American component *X* (positive f_4).

All the results based on *F-statistics* are listed in **S5 Table**. We assessed significance of a comparison considering 3 standard errors (|Z| > 3).

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6 Abbreviations

1KGP: 1000 Genomes Project

AMBA: Metropolitan Area of Buenos Aires

CCP: Central Chile / Patagonia

CWA: Central Western Region in Argentina

CYA: Cuyo Region in Argentina

DS: Data set

LD: Linkage Disequilibrium

LWL: Lowlands

NEA: Northeastern Region in Argentina

NWA: Northwestern Region in Argentina

PCA: Principal Component Analysis

PPA: Pampean Region in Argentina

PTA: Patagonia Region in Argentina

CAN: Central Andes

SNP: Single Nucleotide Polymorphism

YRI: Yoruba individuals in Ibadan, Nigeria

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In memoriam of Raúl Carnese.

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8 Author Contributions

Conceptualization: Pierre Luisi, Angelina García, Darío Demarchi and Hernán Dopazo
Data acquisition: Emma Alfaro, Eliana Aquilano, Carina Argüelles, Sergio Avena, Graciela Bailliet, Julieta Beltramo, Claudio M. Bravi, Raul Carnese, Mariela Cuello, Cristina Dejean, Darío Demarchi, José Edgardo Dipierri, Angelina García, José Luis Lanata, Laura Jurado Medina, Josefina Motti, Maria Laura Parolin, Maia Pauro, Paula Paz, Virginia Ramallo, Daniela Rodríguez Golpe, María Rita Santos, Marisol Schwab, Natalia Silvero
Data curation: Pierre Luisi, Juan Manuel Berros and Jeremias Zubrzycki
Formal analysis: Pierre Luisi
Funding acquisition: Pierre Luisi, Virginia Ramallo and Hernán Dopazo
Investigation:
Methodology: Pierre Luisi, Juan Manuel Berros, Darío Demarchi and Hernán Dopazo

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Project administration: Pierre Luisi and Hernán Dopazo
Resources: Juan Manuel Berros and Hernán Dopazo
Supervision: Hernán Dopazo
Visualization: Pierre Luisi.
Writing – original draft: Pierre Luisi, Angelina García and Hernán Dopazo.
Writing – review & editing: Carina Argüelles, Sergio Avena, Darío Demarchi, Josefina Motti, Maria Laura Parolin, Maia Pauro.

9 Disclosure Declaration

PL provides consulting services to myDNAmap S.A. JMB is employed by Biocódices S.A. HD is the scientific director of Biocódices S.A.

10 Data Availability Statement:

The data analyzed here comprises both newly generated and previously reported data sets. Access to publicly available data sets should be requested through the distribution channels indicated in each published study. For newly genotyped samples, individual genotype data will be made available at http://biocodices.hopto.org/Affymetrix_Pop_Arg/ at time of publication.

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12 Supplementary Figure Captions

S1 Fig. Principal component analysis in a Worldwide Context.
The percentage of variance explained by each principal component (PC) is given. Each point represents an individual following the color and point codes given in legend. **A:** PC2 vs PC1; **B:** PC4 vs PC3; **C:** PC6 vs PC5.

S2 Fig. Admixture Analyses in a Worldwide Context.
(A) Cross-Validation score for Admixture runs on the global meta dataset with *K* from 3 to 12. **(B-G)** Admixture results with *K*=3 to *K*=8. 1KGP: 1000 Genomes Project; CYA: Cuyo Region in Argentina; NEA: Northeastern Region in Argentina, NWA: Northwestern Region in Argentina; PPA: Pampean Region in Argentina; PTA: Patagonia Region in Argentina.

S3 Fig. Comparison of Different Admixture Continental Ancestry Proportion Estimates in a Worldwide Context.
Comparison of the African, European and Native American ancestry proportion estimates obtained with Admixture with *K*=3 and *K*=8. Individuals are represented with points according to the legend of Figure 2. **(A)** African ancestry proportions for K=3 are as observed in green in Supplementary S2B Fig, while for *K*=8 they are estimated as the sum of the three greenish colors observed in S1D-G Figs. **(B)** European ancestry proportions for *K*=3 are as observed in blue in Supplementary S2B Fig, while for *K*=8 they are estimated as the sum of the two bluish colors observed in S1D-G Figs. **(C)** Native American ancestry proportions for *K*=3 are as observed in orange in S2B Fig, while for *K*=8 they are estimated as the sum of the three reddish colors observed in S2D-G Figs.

S4 Fig. Comparison of Different Admixture Continental Ancestry Proportions Among Different Samples.
Comparison of ancestry proportion estimates with admixture (*K=8*) among samples from the present study, and Argentinean, Chilean and Peruvian samples from [33]. **(A)** African Ancestry, **(B)** European Ancestry, **(C)** Native American Ancestry. *P*-value of the Wilcoxon test for each pairwise comparison is shown.

S5 Fig. Comparison of Admixture and RFMix Ancestry Proportion Estimates in DS2p and DS3p.
(A-F) For DS2p: Argentinean samples from the present study with reference panel that consists in 1KGP individuals from Africa, Europe and America [45] and Chilean individuals from [37]. **(A-C)** Native American, European and African ancestry proportions estimates with RFMix vs with Admixture with *K=3*. **(D-F)** Native American, European and African ancestry proportions estimates with RFMix vs with Admixture (*K=7*). **(G-L) For DS3p:** Argentinean samples from [33] with reference panel that consists in 1KGP individuals from Africa, Europe and America [45]. **(G-I)** Native American, European and African ancestry proportions estimates with RFMIx

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vs with Admixture with *K=3*. **(J-L)** Native American, European and African ancestry proportions estimates with RFMIx vs with Admixture with *K=5*.

S6 Fig. Example of a Local Ancestry Output.
(A) RFMIX output for a given admixed individual. **(B)** Masked genotype showing ditypes of Native American (red), European (blue) and African (green) ancestry. Gaps are represented in grey and regions with unassigned ancestry (Unknown) are in black.

S7 Fig. European Ancestry Specific Admixture Analysis.
(A) Cross-Validation scores for *K* from 2 to 10. **(B)** Admixture plots for *K=2*. **(C)** Admixture plots for *K=3*. CYA: Cuyo Region in Argentina; NEA: Northeastern Region in Argentina, NWA: Northwestern Region in Argentina; PPA: Pampean Region in Argentina; PTA: Patagonia Region in Argentina.

S8 Fig. European Ancestry Specific Principal Component Analysis.
(A) Multidimensional scaling based on the weighted Euclidean distance for PCs 1 to 3. Individuals from the European reference panel are colored according to main European geographic regions as shown in **(B)** while South American admixed individuals are represented as shown in the legend**.** Elbow method to choose the number of relevant PCs is shown as inset within (A).

S9 Fig. Choose The Number Of Principal Components From African Ancestry-Specific Principal Component Analysis.
Elbow method to determine which PC minimizes the angle of the curve from the chat "Percentage of variance explained *versus* Number of PCs"

S10 Fig. African Ancestry-Specific Admixture Analysis
(A) Cross-validation scores *K* from 2 to 10. **(B)** Admixture plots for *K=2*. **(C)** Admixture plots for *K=3*. **(D)** Admixture plots for *K=4*. CYA: Cuyo Region in Argentina; NEA: Northeastern Region in Argentina, NWA: Northwestern Region in Argentina; PPA: Pampean Region in Argentina; PTA: Patagonia Region in Argentina

S11 Fig. Choose The Number Of Principal Components From Native American Ancestry-Specific Principal Component Analysis.
Elbow method to determine which PC minimizes the angle of the curve from the chart "Percentage of variance explained *versus* Number of PCs"

S12 Fig. Native American Ancestry-Specific Admixture Analysis
(A) Cross-validation scores *K* from 2 to 10. **(B)** Admixture plots for *K=2*. **(C)** Admixture plots for *K=4*. **(D)** Admixture plots for *K=5*. CYA: Cuyo Region in Argentina; NEA: Northeastern Region in Argentina, NWA: Northwestern Region in Argentina; PPA: Pampean Region in Argentina; PTA: Patagonia Region in Argentina

S13 Fig. Correlation of Native American Ancestry Proportions and Geographical Coordinates in Argentina
(A) Central Andes ancestry proportions vs Latitude. **(B)** Central Andes ancestry proportions vs Longitude. **(C)** Lowlands ancestry proportions vs Latitude. **(D)**

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Lowlands ancestry proportions vs Longitude. **(E)** Central Chile/Patagonia ancestry proportions vs Latitude. **(F)** Central Chile/Patagonia ancestry proportions vs Longitude. Linear regression slopes and the associated *P*-values are shown.

S14 Fig. Comparison of Native American Ancestry Proportion Estimates Obtained with Admixture Run on Unmasked Data (*K=8)* and Masked data (*K=3*).
Spearman correlation coefficients and associated *P*-values are shown. CAN: Central Andes; LWL: Lowlands; CCP: Central Chile / Patagonia.

S15 Fig. Individual Assignation to a Native American Ancestry Cluster.
Consensus clustering South American individuals based on three K-means procedures run with different pairwise distances among individuals. **(Top)** K-means results using Ancestry-Specific PCA and Admixture (ASPCA and AS-Admixture), and f_3 results to compute pairwise distances. Individuals are represented as in **Main Fig 4**. Insets: BIC score for number of clusters set to *K-means* ranging from 2 to 20. In all the three cases, *K*-means BIC was minimized when considering 4 clusters. **(Bottom)** Same as top with point colors corresponding to the assigned cluster.

S16 Fig. Pairwise Genetic Affinity Among Individuals Assigned to Different Groups.
Boxplots of the *1-f3(YRI; Ind1, Ind2),* where *Ind1* and *Ind2* are two individuals belonging to Group 1 and Group 2, respectively. The groups are either the fourth Native American components identified or ancient Southern Cone groups from Middle Holocene. For clarity, boxplot outliers are not shown. YRI: Yoruba from 1KGP.

S17 Fig. Graphical Visualization of Pairwise Genetic Distances Among Groups in South America
(A) Neighbor-joining tree from distances of the form $1/f_3(YRI; X, Y)$. USR1 from Ancient Beringia was used as *outgroup* **(B)** Multidimensional-scaling from distances of the form $1-f_3(YRI; X, Y)$. Each group is represented as appearing in the leaf of (A). USR1 and Anzick-1 were not considered in (B). YRI: Yoruba from 1KGP

S18 Fig. Genetic Affinity of the Four Native American Components with Ancient Groups.
(A-D) *f3(YRI; X; Ancient)*. **(*E-H*)** *f4(YRI, X; Ancient Beringia, Ancient).* **(A)** and **(E)**: with Central Andes (CAN) as *X.* **(B)** and **(F)**: With Lowlands (LWL) as *X.* **(C)** and **(G)**: With Central Chile / Patagonia (CCP) as *X.* **(D)** and **(H)**: With Central Western Argentina (CWA) as *X.* YRI: Yoruba from 1KGP; *Ancient Beringia*: USR1 individual from [73]; *X*: Native American component in Argentina (one plot per *X). Ancient*: ancient group labeled on the *x*-axis and represented with a point/color scheme as in **Main Figure 4**. Vertical segments are the +/- 3 standard errors intervals.

S19 Fig. Changes Across Time of Genetic Affinity of the Four Native American Components with Ancient Groups.

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Each point represents a *f4* score of the form *f4(YRI, X; Ancient Beringia, Ancient)* vs the age of ancient sample, where *X* is one of the four identified Native American components, and *Ancient* is an ancient group. *X* is represented by the color of the square while *Ancient* is represented by the point within the square. The point code of the ancient samples is shown in Fig.4. *Ancient Beringia*: USR1 individual from [73]. **(A)** f_4 vs age of ancient samples from Southern Cone. **(B)** f_4 vs age of ancient samples from Andes. **(C)** f_4 vs age of ancient samples from Southern Cone considering age correction. **(D)** f_4 vs age of ancient samples from Andes considering age correction. Linear regression slopes and the associated *P*-values are shown. CAN: Central Andes; LWL: Lowlands; CCP: Central Chile / Patagonia; CWA: Central Western Argentina; YRI: Yoruba from 1KGP

S20 Fig. Comparison of Genetic Affinity of an Ancient Group to a Native American Component Relative to Another.
f_4(YRI, *Ancient, X, Y)* where *X* and *Y* are two of the four identified Native American components (one plot per *X*-*Y* combination), and *Ancient* is ancient group labeled on the *x*-axis and represented with a point/color scheme as in Main Figure 4. Vertical segments are the +/- 3 standard errors intervals. Note this setting for f_4 statistics is symmetrical when switching *X* and *Y.*

S21 Fig. Comparison of Genetic Affinity of a Native American Component to Another Relative to an Ancient Group.
f_4*(Ancient, X; Y, YRI)* where *X* and *Y* are two of the four identified Native American components (one plot per *X*-*Y* combination), and *Ancient* is ancient group labeled on the *x*-axis and represented with a point/color scheme as in Main Figure 4. Vertical segments are the +/- 3 standard errors intervals. CAN: Central Andes; LWL: Lowlands; CCP: Central Chile / Patagonia; CWA: Central Western Argentina; YRI: Yoruba from 1KGP

S22 Fig. Removing Admixed Santiago de Chile Individuals from Analyses Does Not Affect the Results.
Admixed individuals from Santiago de Chile were removed to perform the analyses presented in this figure. **(A)** f_3(*Target; S1, S2*) to test for treeness; **(B)** f_4*(YRI, Target; S1, S2)* to test whether Target shares more ancestry with S1 or S2; **(C)** *f3(YRI; CWA; Ancient)*; **(D)** *f4(YRI, CWA; Ancient Beringia, Ancient);* **(E)** f_4*(Ancient, CCP; CWA, YRI);* **(F)** f_4*(Ancient, CWA; CCP YRI);* **(G)** *f4(YRI, Ancient; CWA, CCP).* CCP: Central Chile / Patagonia.

S23 Fig. Schematic Routes for the Main Population Arrivals in the Southern Cone.
Each arrow represents one of the four components discussed throughout the article. Neither disentangling the time and place of the splits among these components nor the extent of potential gene flow have been addressed in this study.

S24 Fig. Admixture Analyses in DS2 to Define European, African and Native American Reference Individuals for Local Ancestry Analyses
(A) Cross-validation scores from *K*=3 to *K*=10. **(B)** Admixture plot for *K=7*

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S25 Fig. Admixture Analyses in DS3 to Define European, African and Native American Reference Individuals for Local Ancestry Analyses
(A) Cross-validation scores from *K*=3 to *K*=10. **(B)** Admixture plot for *K=5*

13 Supplementary Table Captions

S1 Table. Sample information.
Sampling location, Gender, uniparental lineages, Affymetrix QC metrics, Color and Point coding used for plots throughout the article.

S2 Table. Data sets (DS) used throughout the article.

S3 Table. Ancestry proportion estimates in a worldwide context.
Ancestry proportion estimates from Admixture analyses with *K=3* and *K*=8 at the worldwide level. The column names describe the labels attributed to each ancestry detecting for both Admixture analyses, as well as the hexadecimal code for the color used to represent it in the corresponding admixture plot. The columns "Point", "Color" and "cex" list the graphic parameters used to represent each individual in the different plots throughout the article.

S4 Table. Native American cluster assignation.
Individual Native American cluster assignation is given for each of the *K*-means procedures and for the consensus call (columns "F3", "PCA", "Admixture" and "Consensus"). The ancestry proportion estimates from Admixture analyses with *K=3* on the masked data for Native American ancestry are also provided. The column names explicit the labels attributed to each ancestry detecting for both Admixture analyses as well as the hexadecimal code for the color. The column "Argentinean describes whether an individual was used for Argentinean territory specific analyses or not. For admixed individuals (from the present study and [33]), the "Population" and "Region" columns list the locality and province, respectively, while for Native American population (from [37,38])) the "Population" and "Region" columns list the ethnic and main ethnic groups, respectively.

S5 Table. *F*-statistics results
(A) *f3(Target; S1, S2)* only for comparisons including Native American components **(B)** *f4(YRI, Target; S1, S2)* only for comparisons including Native American components **(C)** *f3(YRI; X, Y)* only for comparisons including Ancient Beringia, Mixe and Native American components **(D)** *f3(YRI; X, Y)* where *X* and *Y* can be either an ancient group or one of the four Native American components. **(E)** *f4(YRI, X; Ancient Beringia, Ancient)* only for comparisons including between a Native American components (*X*) and an ancient group (*Ancient*). **(F)** *f4(YRI, Ancient, X, Y)* only for comparisons including two Native American components (*X, Y*) and an ancient group (*Ancient*). **(G)** *f4(Ancient, X; Y, YRI)* only for comparisons including two Native American components (*X, Y*) and an ancient group (*Ancient*). YRI: Yoruba from 1KGP

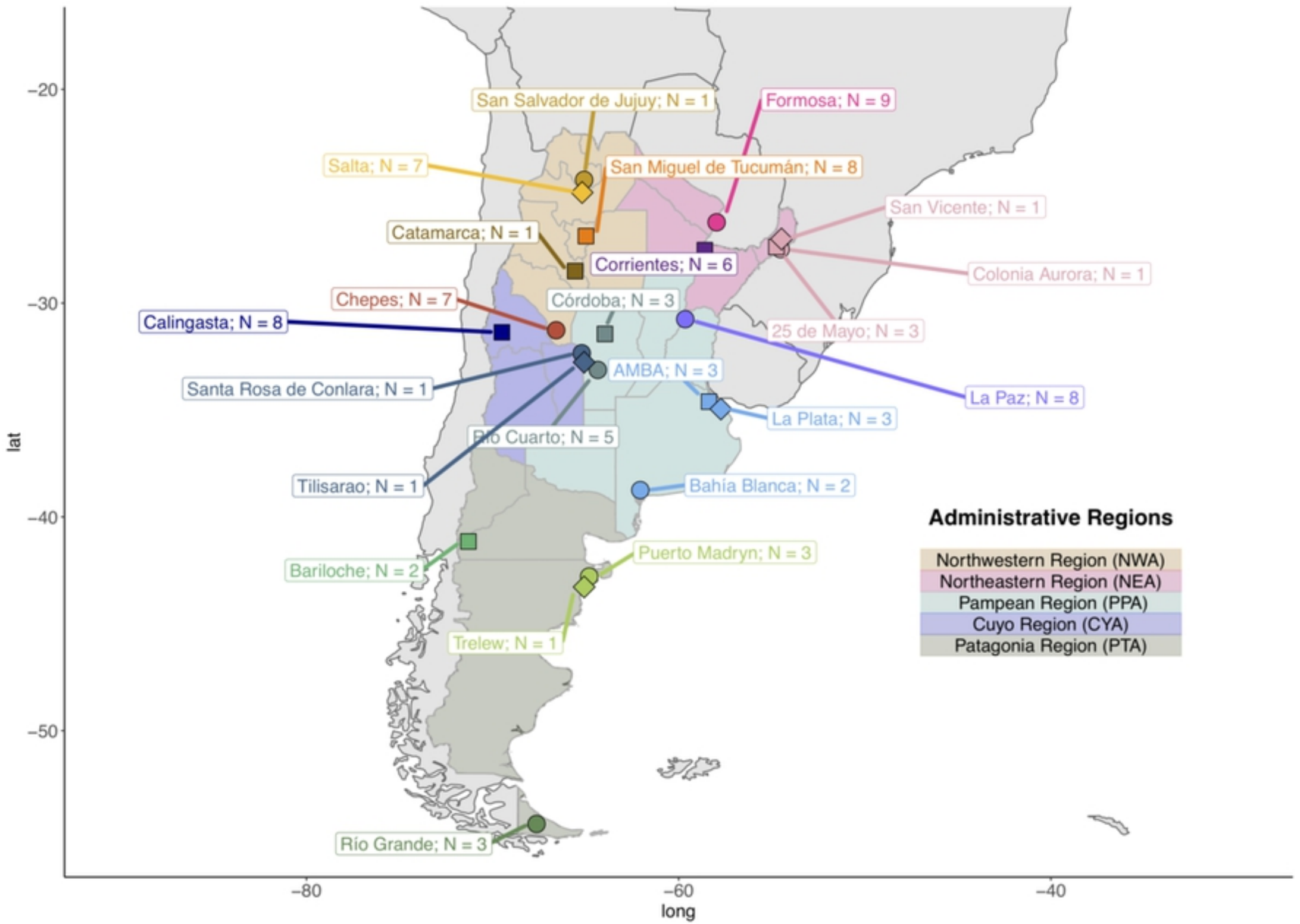

Fig 1. Sample locations from the present study

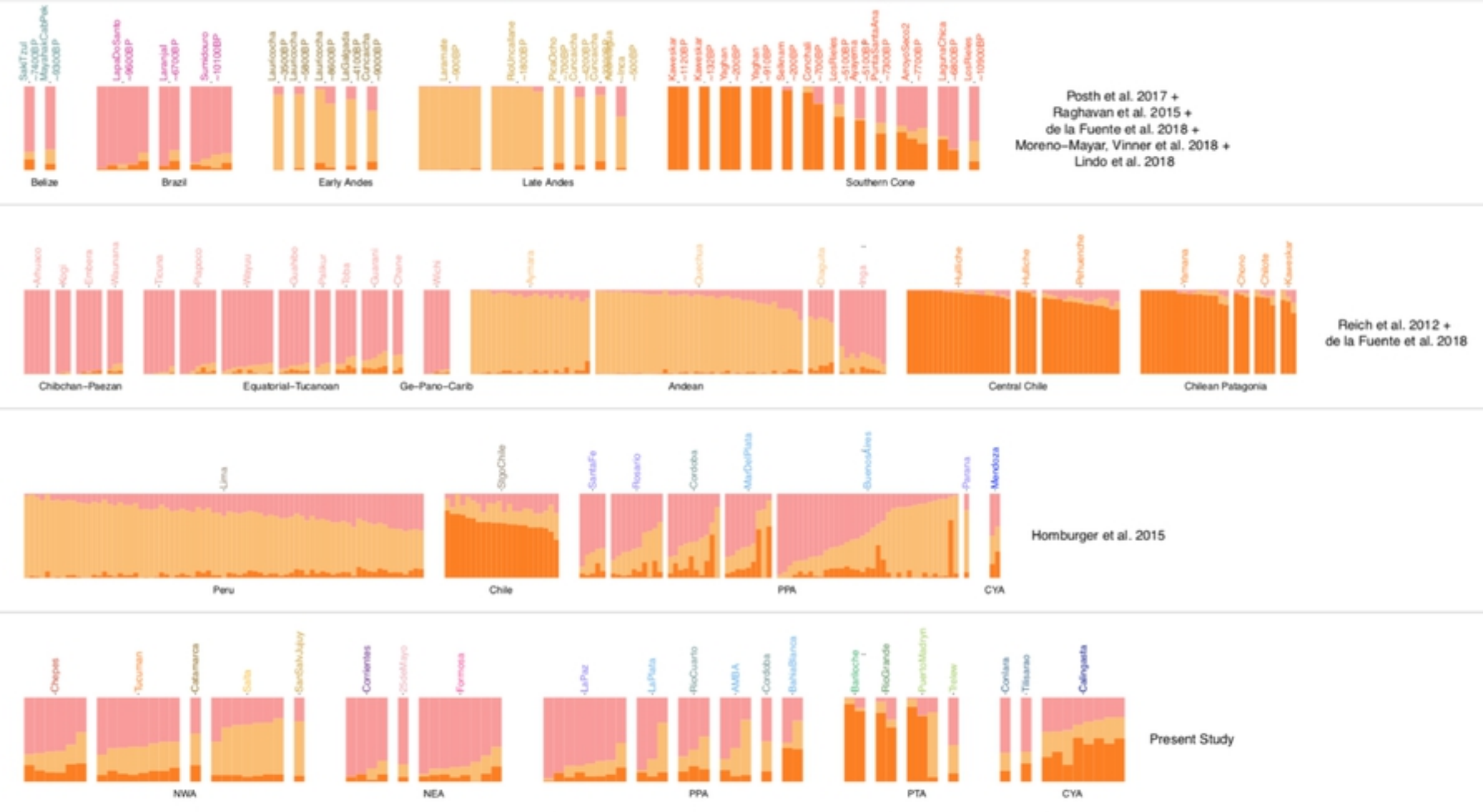

Fig 5. Native American Ancestry-Specific Admixture Analysis.

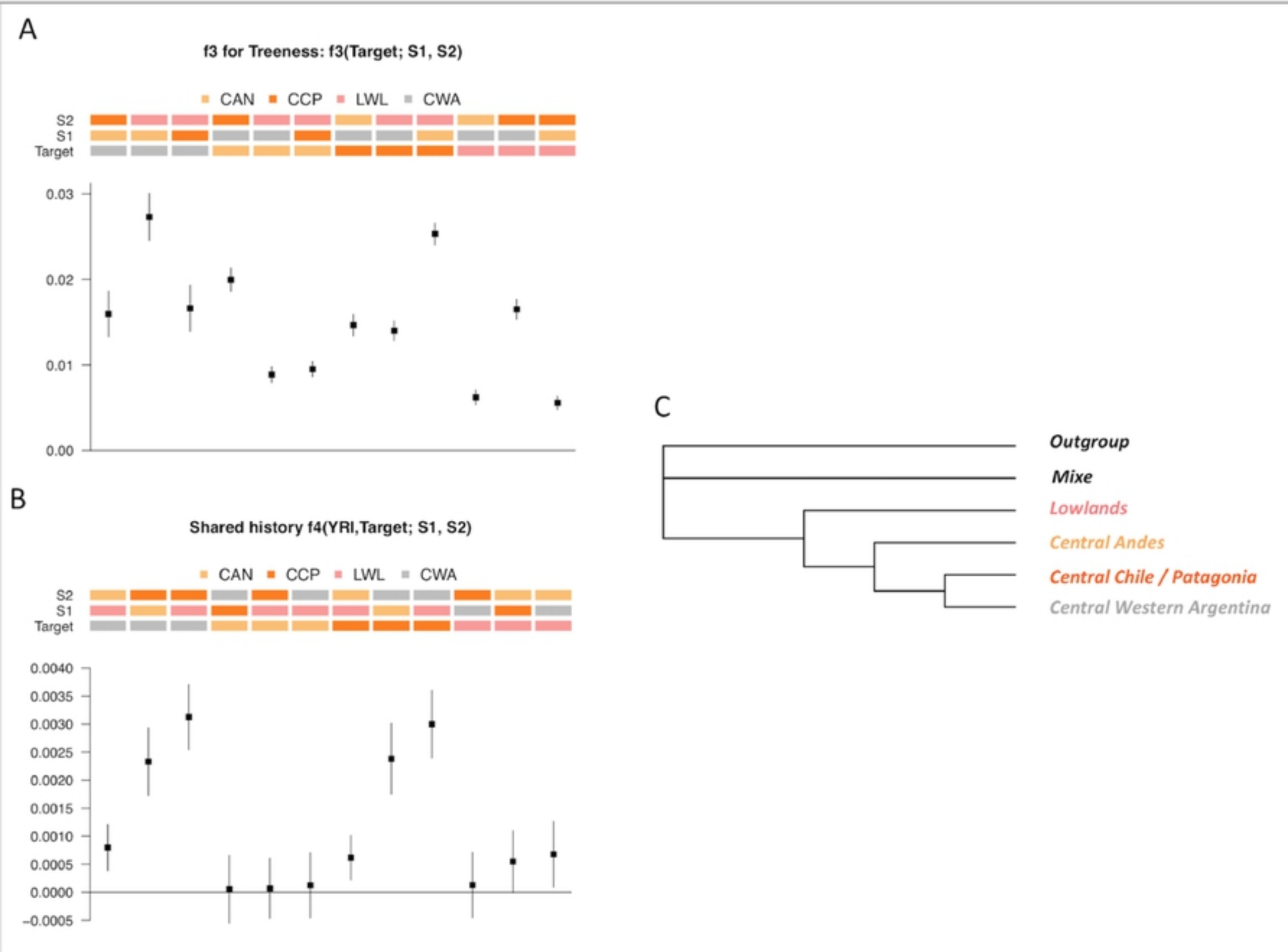

Fig 6. Relationship among the four Native American groups iden

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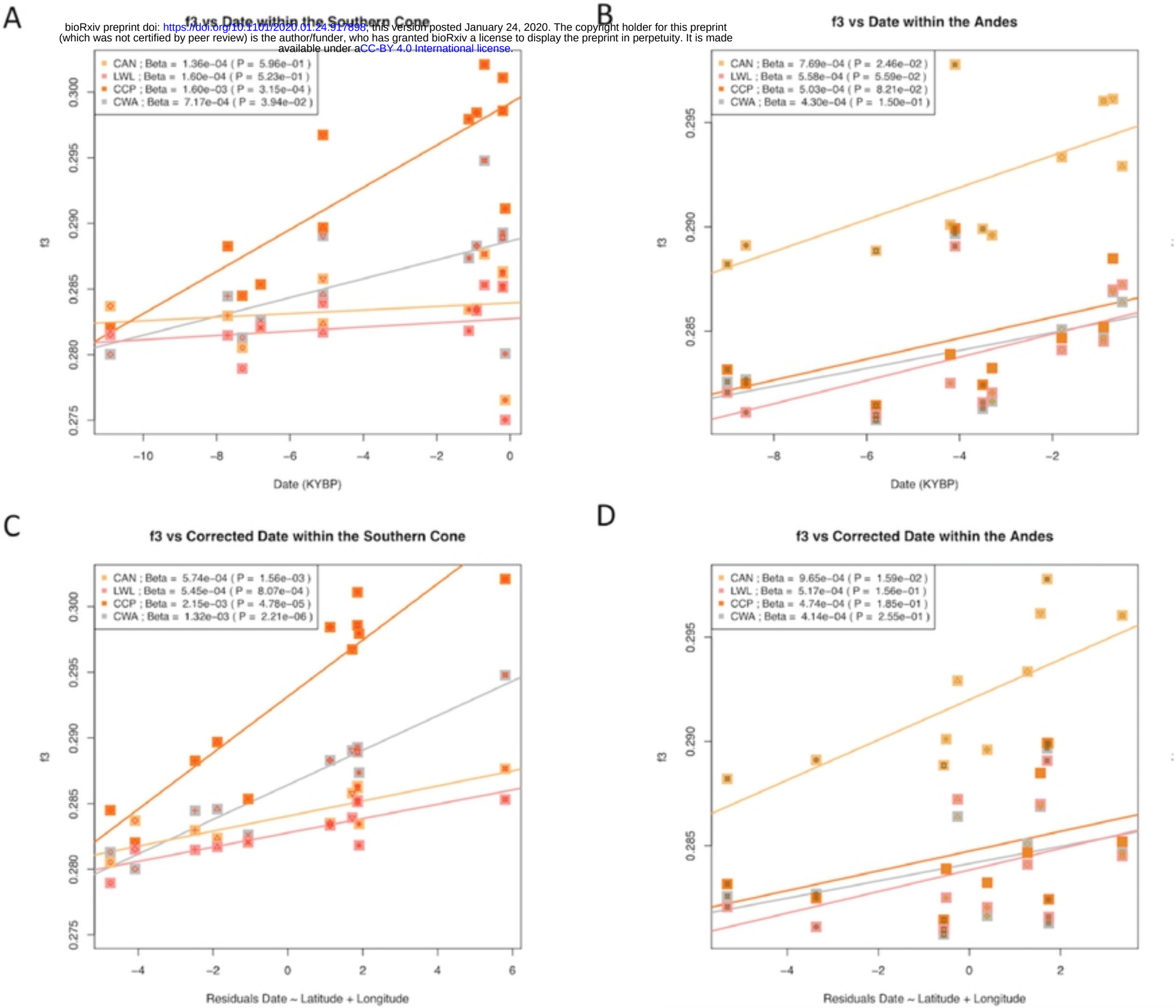

Fig 7. Changes across time of genetic affinity of the four Native A

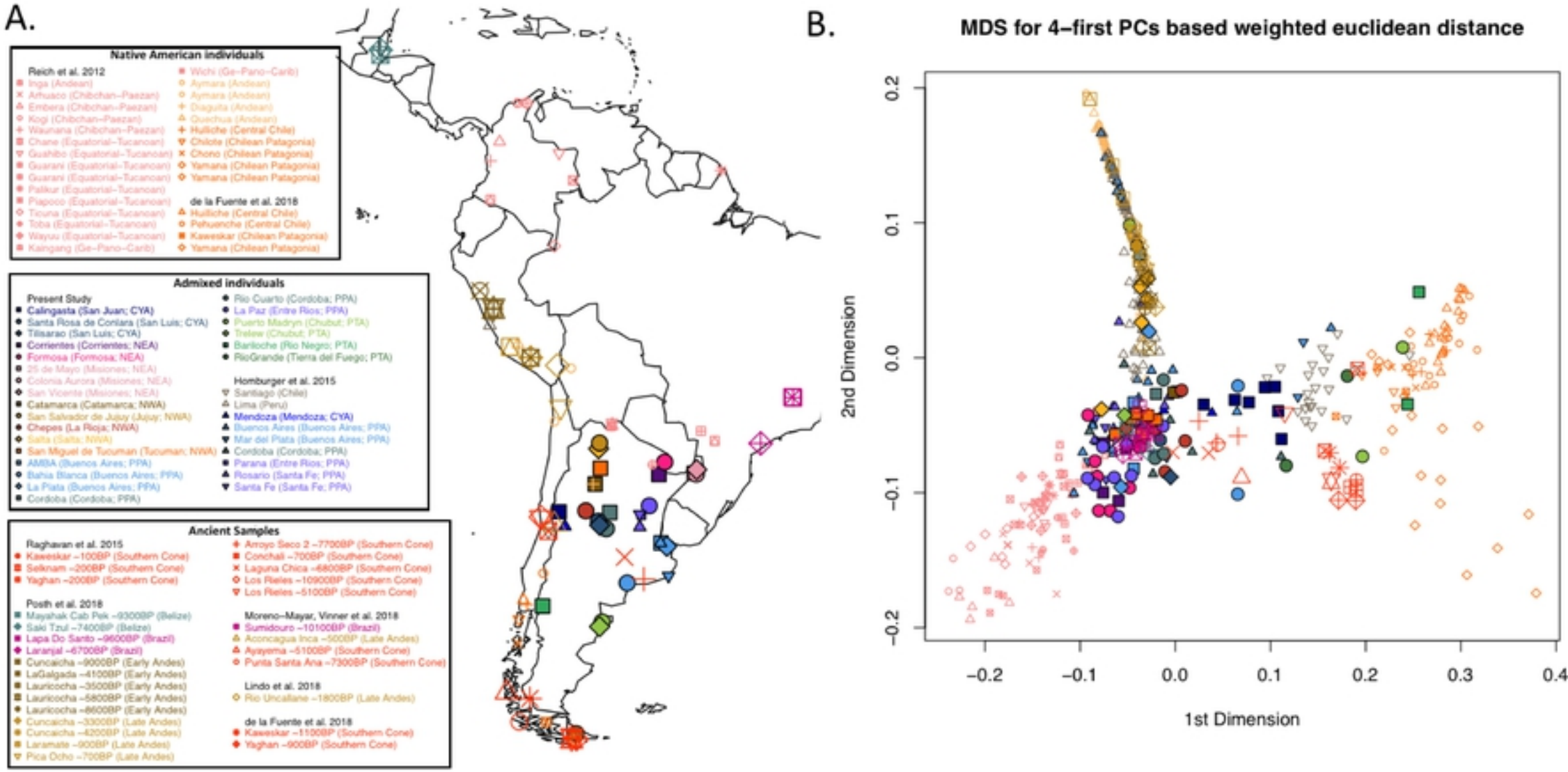

Fig 4. Native American Ancestry-Specific Principal Component An

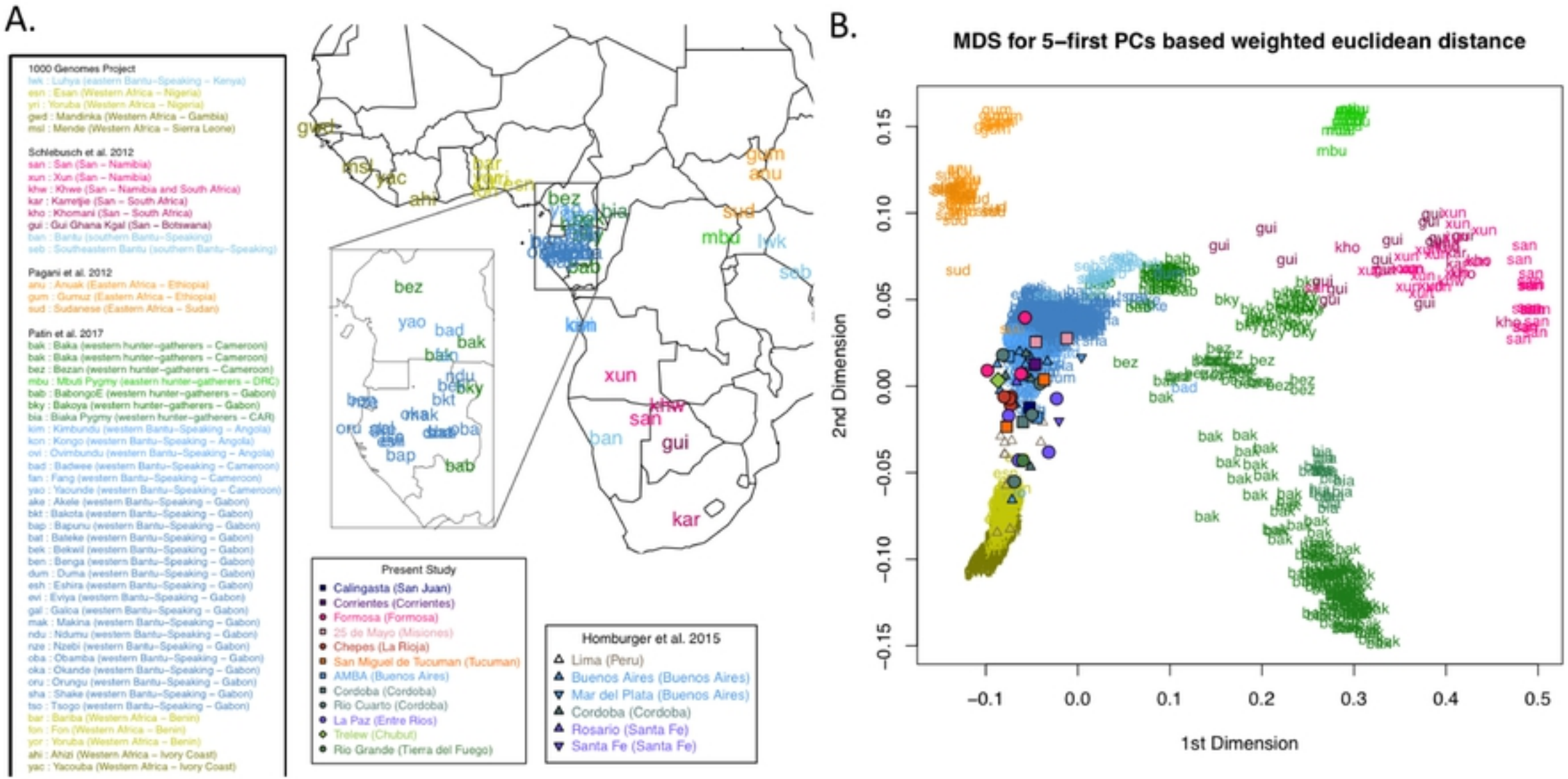

Fig 2. African Ancestry-Specific Principal Component Analysis.

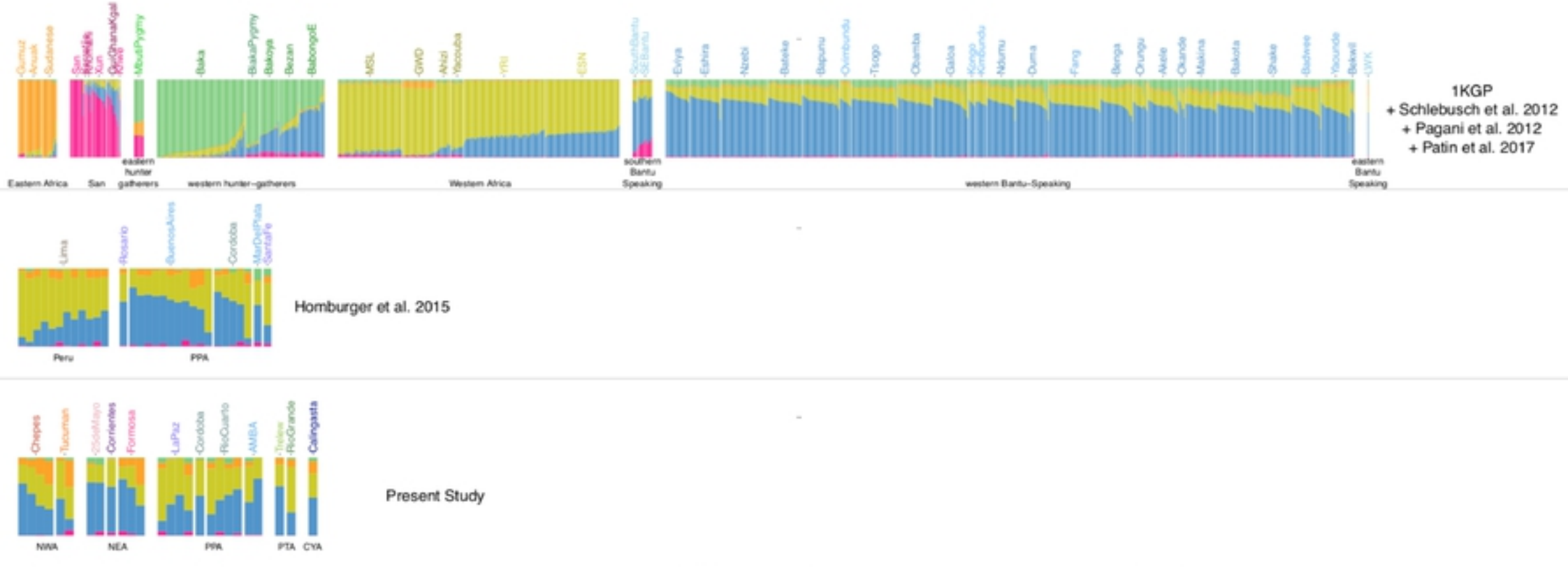

Fig 3. African Ancestry-Specific Admixture Analysis.