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Genetic Admixture Analysis in the Population of Tacuarembó-Uruguay Using Alu Insertions

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Abstract

Tacuarembó is a department located in the northeastern of Uruguay, whose population is the result of several migration waves from Europe and Near East as well as Africans and Afro-descents mostly from Brazil, these waves settled on the territory of various Native ethnic groups (Charrúa, Minuán, and Guaraní). In the past, this population has been the focus of genetic studies showing this tri-hybrid origin, with greater contributions of Natives and Africans than other Uruguayan regions. In this paper we aim to analyze *Alu* insertions (A25, ACE, APOA1, B65, D1, F13B, PV92, TPA25) in order to provide valuable information for ancestrality and genetic differentiation (GD), and to compare with previous studies on Tacuarembó population as well as to *Alu* frequencies in other Uruguayan populations. The European contribution with *Alu* and classical markers (CM) was almost equal to a previous study using 22 classical markers (63% vs 65%) while African contribution was higher (30% v*s* 15% respectively), and the Native Americans contribution shows an important difference 7% in *Alu* v*s* 20%. There are no significant differences between Tacuarembó and Montevideo in GD, but there are significant differences between Tacuarembó and Basques descendants from Trinidad. Our results support the previous findings obtained with classical markers that demonstrate the tri-hybrid composition of the Tacuarembó population, correlated with the historic records. Finally, *Alu* insertions provides interesting information in light of the admixture process in the Uruguayan population.

The department of Tacuarembó (political division) located in the northeast of Uruguay (31°42'S, 55°59'W) occupies 38,730 km2, which represents 22% of the national territory, being sparsely populated with a population of about 93.197 individuals (Instituto Nacional de Estadistica 2014). The region is interesting from the point of view of population genetics since its territory was populated by different Native American groups (Charrúas, Guenoa-Minuanes and Guaranies) (Bracco 2004), its main city (also named Tacuarembó) was founded in 1832 with families that came from Montevideo, following orders of the Uruguayan President Rivera. The selected place was known as "Rincón de Tía Ana", land that had belonged to the free African Ana Josefa Barbera and her Paraguayan husband (Vázquez 2013). The region has received different migratory waves of individuals of Europe and the Near East entering the country from Montevideo harbour, and Portuguese entering from the Brazilian border, as well as Africans or African descendants mostly fleeing from Luso-Brazilian slavery (Sans 1994).

Different approaches have been made in admixture studies of Tacuarembó population, ranging from the study of morphological traits (Mongolian spot, shovel-shaped incisors) (Sans 1994), classical markers (blood groups, serum proteins and HLA system) (Sans et al. 1997) and uniparental inherited DNA (mitochondrial and Y chromosome DNA) (Bonilla et al. 2004; Bertoni et al. 2005). These different approaches have provided interesting results to understand the genetic composition and demonstrate the trihybrid status of the population formed by a mixture of Europeans, Native Americans and Africans.

Analyses performed using classical markers (CM) showed a European contribution of 65%, 20% Native American and 15% African (Sans et al. 1997). Mitochondrial DNA analyses have revealed that the main contribution through the maternal line is the Native

American with 62% (Bonilla et al. 2004) while the study of the Y chromosome showed an almost exclusive European contribution for the paternal line with 1.6 - 8.31%

Native American, although it was not possible to detect the African contribution (Bertoni et al. 2005).

At present, no autosomic DNA markers were analyzed in the population Tacuarembó. Consequently, we have decided to study the *Alu* insertion frequencies since they provide valuable information of genetic structure and evolutionary history of populations (Perna et al. 1992). They are robust markers for microevolutionary studies because they have a unique mutational mechanism, an absence of back mutations, and a lack of recurrent forward mutations (Okada 1991; Batzer et al. 1994; Hamdi et al. 1999; Roy-Engel et al. 2001). Specific *Alu* insertions and nearby flanking sequences will be identical by descent in all individuals in whom they occur (Batzer et al. 1994).

Alu insertions are short interspersed elements (SINES) that represent the largest family of movile elements in the human genome (Batzer et al. 2002). They have been studied widely in large continental groups since they generate valuable information for studies of admixture and genetic differentiation of human populations. In Uruguay *Alu* insertions have allowed to make approximations to understand the process experienced by the Basques, one of the major migration waves that populated that country, and their relation to other immigrants, as well as to Native American and African descendants (Hidalgo et al. 2014).

Our study is focused in analyzing the intra e inter populational variability and estimating the genetic contribution of Tacuarembó population using the allelic frequency of 8 *Alu* insertions (A25, ACE, APOA1, B65, D1, F13B, PV92, TPA25) and compare that results with previous admixture studies performed with classical markers as a way to provide information to dilucidate microevolutionary processes as well as historical facts.

Materials and Methods

Blood samples were collected from 120 unrelated individuals of Tacuarembó department. Ethical aspects were approved by the Facultad de Humanidades y Ciencias de la Educación-Universidad de la República (UdelaR)-Ethics Review Board and informed consent was obtained from all participants.

DNA Typing and PCR

The genetic characterization of the Tacuarembó population was carried out by analyzing 8 *Alu* insertions (A25, ACE, APOA1, B65, D1, F13B, PV92, TPA25), typed using DNA isolated from peripheral venous blood and saliva. DNA was extracted from blood with QIAamp DNA Mini Kit, for saliva samples was used the extraction protocol used in Facultad de Medicina, UdelaR (B. Bertoni, Personal communication). The 8 *Alu* insertions were typed according to the PCR conditions previously established by García-Obregón et al. (2006). Genotyping was carried out through electrophoresis in 2% agarose gels and staining with ethidium bromide; gels were visualized under ultraviolet light. In some cases, the results were confirmed by polyacrylamide gel electrophoresis with silver staining.

For the genetic affinity analysis of Tacuarembó, 19 selected European, African descendants, and Native American populations and three populations from Uruguay were used (Table 1).

Statistical Methods

Allele and genotype frequencies of *Alu* insertions were obtained by direct gene counting (Li 1976). The Hardy-Weinberg Equilibrium (HWE) was determined at each locus by chi-square test using GenAlEx program version 6.5 (Peakall and Smouse 2012) and with an exact test using the Biosys 2 program (Swofford and Sealander 1997). The heterogeneity in allele frequencies among Uruguayan samples was analyzed with an exact test using the Genepop

4.2.2 package (Rousset 2008). Genetic differentiation was measured by Gst (Nei 1987) using the gene diversity for the entire population (Ht) and the average gene diversity for samples (Hs) with the Dispan computer program (Ota 1993).

Nei's DA distance (Nei et al. 1983; Nei 1987) was computed between the Uruguayan samples and the populations listed in table1. DA genetic distances were represented by unweighted pair group method with arithmetic mean (UPGMA) trees (Sneath and Sokal 1973) by means of the Neighbor program, generating 1,000 bootstrap replicates with Seqboot, and a consensus tree was obtained with Consense as implemented in the PHYLIP package (Felsenstein 2002).

The Euclidean distances, proximity matrix and graphical representation of the multidimentional scaling (MDS) were made with XLSTAT (2017) program.

Genetic admixture was estimated by gene identity method (Chakraborty 1975, 1986) using the average of allele frequencies for the European, African descendants, and Native American populations listed in Table 1. The allele frequencies for each individual locus were pooled and weighted by arithmetical means.

Results

Table 2 shows the allele frequencies of 8 *Alu* insertions analyzed in Tacuarembó. The highest allele frequency corresponds to the APOA1 insertion. The lowest frequencies were observed for the A25 insertion.

Gene diversity given in Table 2 shows that the intra-populational gene diversity (Hs) ranges from 0.2077 (APOA1) to 0.4593 (TPA25), with a mean value of 0.3481. The gene differentiation coefficient relative to the total population (Gst) shows large variation from locus to locus (0.0713–0.2952), with the highest differentiation for the PV92 and the least for

TPA25. The mean Gst over all 8 loci is 17 % indicates differentiation in *Alu* insertions. Both the Hs and the Gst were calculated including the Uruguayan populations indicated in Table 1.

The Hs for each of the populations selected show that for the group of African populations it goes from 0.305 (Nguni) to 0.468 (Kenya), for European populations range from 0.322 (Italians) to 0.387 (Basques), and 0.127 (Waorani) to 0,299 (Xavante) for Native American group. On the other hand, Uruguayan populations range from 0,342 (BMTV) to 0.379 (BTD) (Table. 3). Significant deviations from HWE were observed after Bonferroni correction for APOA1, ACE and F13B insertions (Table 4).

The study of genetic heterogeneity for 8 *Alu* insertions for each paired sample from Uruguay was calculated. Tacuarembó exhibited significant differences with respect to Basque descendants from Trinidad but did not show significant difference with Montevideo or Basque-descendants from Montevideo (Table 4). Genetic distances based on Nei's DA range from 0.21% to 3.34%, with the highest value between Tacuarembó and Basque descendants from Trinidad. (Table 5). Nei´s distances between Tacuarembó, Montevideo and Basque descendants from Montevideo samples are not significant ($p>0.05$), but the distances between Tacuarembó and Basque descendants from Trinidad are statistically significant (($p<0.05$).

Using gene identity method (Chakraborty 1975, 1986), we estimated in Tacuarembó an average of 0.07 ± 0.02 Native contribution and 0.3 ± 0.10, African contribution. The level of admixture estimated indicates a substantial European ancestry in the sample with 0.63 ± 0.10. The results were compared with previous admixture studies of Tacuarembó using classical markers (Sans et al. 1997) (Table 7).

The UPGMA tree (Figure 1) shows that the Uruguayan samples are placed in the cluster formed by the European populations and separated from the clusters formed by Africans and by Native Americans. However, Montevideo samples (MVD and BMVD) and

Tacuarembó (TBO) appear separate within the European cluster, while the Basque descendants from Trinidad (BTD) sample is more related to the Spanish populations.

The multidimentional scaling analysis (Figure 2), shows groupings of the main geographic origins; Africans, Europeans and Native Americans, being the Uruguayan populations a small group near Europe. similar to the UPGMA tree, BTD sample is closer to Basques and Andalusians in the European group than the other Uruguayans populations, while Tacuarembó is closer to MVD and BMVD. Besides, African Americans (AAM) appear closer to Uruguayans than the Africans.

Discussion

For more than three decades, admixture studies in Latin America have aroused significant interest in researchers, from the studies of morphological traits to the most recent analyzes performed with SNPs. They have contributed to the reconstruction of the historical and anthropological processes of the last five hundred years. A large number of studies have been carried out using different approaches, but in summary they show that the region is made up for at least a mixture of Native American, European and African populations, which largely reflects the documented history (Salzano and Sans 2014).

Uniparental DNA studies in Tacuarembó had showed 95 % European paternal line (Bertoni et al. 2005) but 62% Native American maternal line (Bonilla et al. 2004). This pattern was commonly observed in Latin Americans, as a consequence of directional matings between European males and Native females (Boyd-Bowman 1976; Carvajal-Carmona et al. 2000). To complement the previous studies of uniparental and classical markers, 8 *Alu* insertions were analyzed in Tacuarembó, showing similar frequencies to those previously recorded in Montevideo and Basque descendants from Montevideo, but different to those from Basque descendants from Trinidad, in the southcentral region of Uruguay. Three *Alu*

insertions, APOA1, ACE and F13B showed significant departure from HWE after Bonferroni correction. Possible explanations could be nonrandom mating, recent migrations, and substructures (o subpopulations) generated because of recent admixture (Schaid et al. 2006).

Our estimations of *Alu* insertions show that the ancestral contribution that predominates in Tacuarembó is the European with 63%, previous investigations with classic markers show similar results with 65% (Sans et al. 1997). The African contribution occupies the second place with 30 % in the results of *Alu* insertions, but in terms classic markers it occupies third place with 15% (Sans et al. 1997). The indigenous contribution for the *Alu* insertions was the smallest contribution with 7% while for the classical markers the indigenous contribution was 20% occupying the second place (Sans et al. 1997).

Then, these results show a significant increase of the African contribution when the *Alu* insertions were studied reaching a 30% which represents twice the contribution obtained with classical markers. A similar pattern was observed in Montevideo, where African contribution reached 15% (Hidalgo et al. 2014) versus 7% when classic markers were studied (Sans et al. 1997). Otherwise the Native American contribution calculated with *Alu* insertions in Tacuarembó show a decrease respect to the one estimated by classical markers (7% vs 20% respectively), decrease not noted in Montevideo samples.

The African contribution found in Tacuarembó with *Alu* insertions can be related with the fact that the Tacuarembó department is next to Rivera department, this latter, according to the 2011 census (INE 2011), is the Uruguayan department with the highest average number of self-defined people as Afro-descendants (17.3%), while Tacuarembó is in fourth place of self-defined people as Afro-descendants with 9.9% (Cabella et al. 2013).

Previous studies conducted in Argentine Patagonian population, show that when carrying out admixture studies using classical markers (blood systems) and *Alu* insertions (16 loci) separately, the results are consistent in both estimation (Parolin et al. 2017). Possible

explanations to differences in found in Uruguay using different types of markers can be discussed on the following bases: a) *Alu* insertions in Uruguayan populations (Montevideo and Tacuarembó) with respect to the previous studies using classical markers, can be more informative for the African component and less informative for Native American contribution in Tacuarembó or they can sub or overestimate the ancestral contributions mentioned above; b) the chromosomes of people living in the present show a complex mix of ancestry, containing blocks of different ancestry depending on the number of generations of admixture, marriage patterns, and the characteristics of the mixed people involved in them (Salzano and Sans 2014). That factor could affect the estimations of admixture when different genetic autosomal markers are compared; c) different factors such as sampling, *Alu* insertions selected, lack of adequate data on parental populations for the estimates, and/or changes in the frequencies in the parental populations over time (Hidalgo et al. 2014). Another reason for explaining these results could be the low number of *Alu* insertions analyzed.

Notwithstanding the above, *Alu* insertions analysis is a complementary tool for population genetic analysis, being a simple and economical molecular technique, however it becomes necessary to increase the biparental genetic information including a greater number of bi-parental DNA markers such as *Alu* insertions or AIMs (Ancestry Informative Marker) in order to obtain robust results.

Finally, it is necessary to mention that this study is the first genetic analysis and admixture estimations in a population of the northeast of Uruguay using autosomal DNA markers, these results allow to support the previous findings obtained with classical markers that demonstrate the trihybrid composition of the Tacuarembó population, correlated with the historic records. They also increase the genetic knowledge applied to the historical, anthropological and biomedical fields of Uruguayan and Latin American populations.

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Table 1. Populations Selected for the Genetic Affinities Analyses

Region/Populations	N	Code	Reference
Europeans			
Catalans	60	CAT	Comas et al., 2000
Andalusians	67	AND	Comas et al., 2000
Galicians	73	GAL	Terreros et al., 2009
Basques	96	BAS	Comas et al., 2000
French	72	FRE	Romualdi et al., 2002
Italians	263	ITAL	Selvaggi et al., 2010
Africans			
Benin	40	BEN	Terreros et al., 2009
Kenya	40	KEN	Terreros et al., 2009
Nguni	44	NGU	Romualdi et al., 2002
Rwanda	46	RWA	Terreros et al., 2009
Cameroon	16	CAM	Terreros et al., 2009
Bantu	48	BAN	Romualdi et al., 2002
USA			
African Americans	69	AAM	Romualdi et al., 2002
Native Americans			
Guarani	35	GUAR	Battilana et al., 2002
Maya	28	MAY	Romualdi et al., 2002
Waorani	36	WAO	Gómez-Peréz et al., 2011
Caingang	48	CAING	Battilana et al., 2002
Xavante	33	XAVAN	Battilana et al., 2002

Aché	76	ACHE	Battilana et al., 2002
Uruguayans			
Montevideo	70	MVD	Hidalgo et al., 2014
Basques from MVD	55	BMVD*	Hidalgo et al., 2014
Basques from Trinidad	58	BTD*	Hidalgo et al., 2014

*Samples from individuals of self-defined Basque descent.

Table 2. Allelic Frequency in Tacuarembó, Heterozygosity, and Gene Diversity Analysis

Alu	Chromosomal position	N	Allelic frequency	Ht*	Hs*	Gst*
A25	8q21.3	119	0.071	0.2572	0.2260	0.1215
ACE	17q23	112	0.321	0.4935	0.4397	0.1089
APOA1	11q23-24	116	0.849	0.2548	0.2077	0.1847
B65	11q14.2	116	0.241	0.4904	0.4297	0.1237
D1	3q26.32	110	0.195	0.4545	0.3756	0.1734
F13B	1q31-32.1	100	0.435	0.4808	0.3422	0.2883
PV92	16q24.2	114	0.224	0.4323	0.3047	0.2952
TPA25	8p11.2	120	0.331	0.4946	0.4593	0.0713
Median	-----	113	0.333	0.4198	0.3481	0.1706

*Were calculated including the Uruguayan populations.

Table 3. Intrapopulational Gene Diversity (Hs) of Populations Selected

Population	Heterozygosity	Standard error
Tacuarembó	0.346244	0.046560
Montevideo	0.357828	0.042660
Basques descendants from Montevideo	0.342420	0.038652
Basques descendants from Trinidad	0.379643	0.059425
Catalans	0.344671	0.065129
Andalusians	0.357996	0.066239
Galicians	0.323778	0.068231
Basques	0.387394	0.058079
French	0.342266	0.062163
Italians	0.322670	0.073071
Benin	0.427886	0.027524
Kenya	0.468407	0.011897
Nguni	0.305471	0.065492
Rwanda	0.385415	0.050061
Cameroon	0.438435	0.020494
Bantu	0.308730	0.063967
African Americans	0.366283	0.065232
Maya	0.265047	0.071141
Waorani	0.127340	0.069035
Guarani	0.276925	0.051444
Aché	0.204560	0.074731
Caingang	0.273323	0.059949

Xavante	0.299579	0.081682

Table 4. Hardy Weinberg Equilibrium Tests

Locus	ChiSq p-value	Exact Test p-value	Bonferroni Correction
APOA1	0.000	0.000	0.000*
ACE	0.000	0.001	0.008*
TPA25	0.083	0.101	0.808
PV92	0.075	0.103	0.824
A25	0.001	0.012	0.096
B65	0.032	0.040	0.320
F13B	0.001	0.001	0.008*
D1	0.021	0.030	0.240

*highly significant.

Table 5. Genic Differentiation for Each Population Pair across All Loci (Fisher's Method)

Population pair	Chi2	*df*	p-Value
TBO – MVD	21.819	16	0.14911
TBO – BMVD	22.705	16	0.12184
TBO – BTD	0.1×10^5	16	0.0000 *

*highly significant.

Table 6. Nei's DA Genetic Distance of Tacuarembó and Uruguayan Populations

	TBO	MVD	BMVD	BTD
TBO	****			
MVD	0.0021	***		
BMVD	0.0036	0.0052	***	
BTD	0.0334	0.0189	0.0433	***

Table 7. Genetic Admixture Analysis of Tacuarembó Population

	Alu insertions (This study)	Range	Classical Markers (Sans et al. 1997)	Range
European	0.63	0.53 – 0.73	0.65	0.60 – 0.77
African	0.30	0.20 – 0.40	0.15	0.11 – 0.19
Native American	0.07	0.05 – 0.09	0.20	0.18 – 0.25

Figure Captions

Figure 1. UPGMA tree in Uruguayan and parental populations.

Figure 2. Multidimentional Scaling Analysis (MDS). Tacuarembó and parental populations. Configuration (Kruskal's stress = 0,107).

Figure 1.

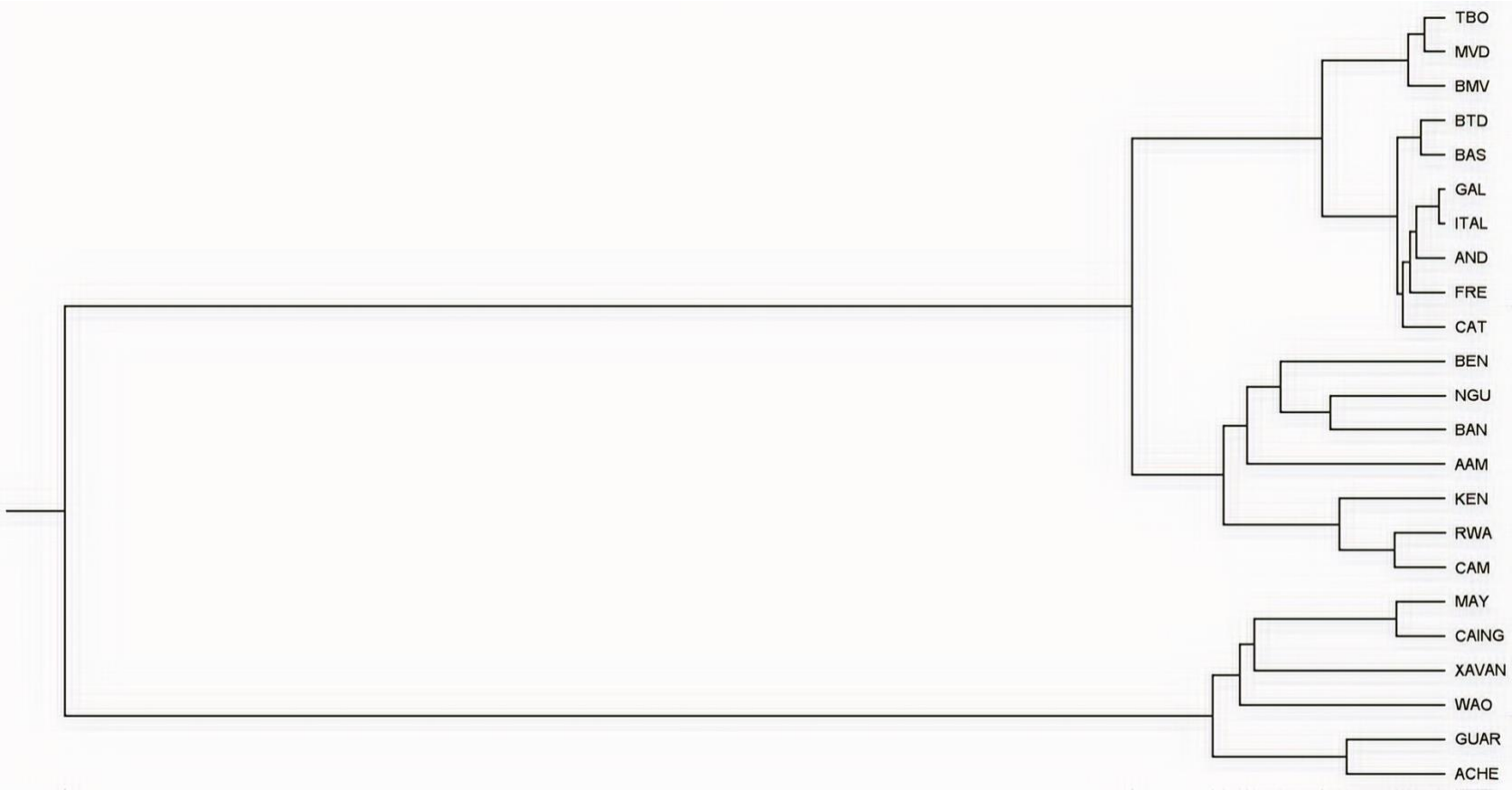
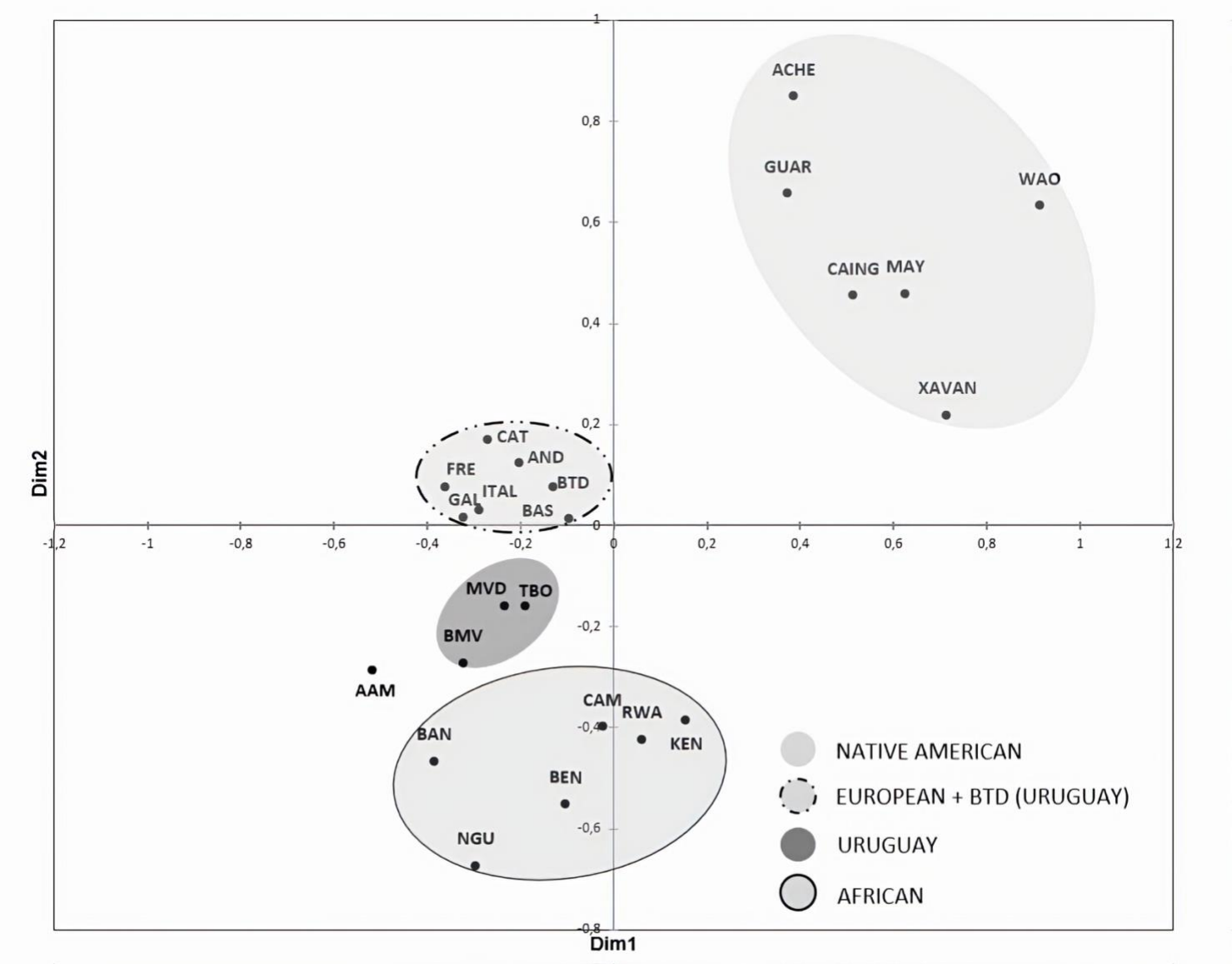


Figure 2.


ACHE
GUAR
WAO
CAING
MAY
XAVAN
CAT
AND
FRE
BTD
ITAL
GAL
BAS
MVD
TBO
BMV
AAM
CAM
RWA
KEN
BAN
BEN
NGU
NATIVE AMERICAN
EUROPEAN + BTD (URUGUAY)
URUGUAY
AFRICAN
Dim1
Dim2