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The "last Charrúa Indian" (Uruguay): analysis of the remains of Chief Vaimaca Perú.

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Uruguay is the only Latin American country that at present lacks Native populations and little is known about its prehistoric populations. In the construction of National identity, the unique reference to Natives is about Charrúa Indians, one of the most important ethnic groups that lived in the territory and exterminated in the 1830s. In 1833, four survivors were taken to be exhibited and studied in France, becoming martyrs and a symbol of their nation. The skeletal remains of Chief Perú were preserved and studied mainly by Rivet[1]; these are the only remains certainly identified as belonging to a Charrúa. In 2002, the French government returned the remains to Uruguay, where they were buried with honours at the National Pantheon. Before the burial, we performed morphological studies as well as extracted samples for DNA analysis. Peru's morphology is coherent with the one of a nomadic warrior: robust body with strong muscular insertions, wounds, and healthy diet based mainly on meat. Here we show that metric and morphological data as well as maternal inherited mitochondrial DNA (mtDNA) hypervariable region I (HVRI) and restriction fragment length polymorphisms (RFLPs), indicate a close relationship with Pampa-Patagonian Indians, and specially, with prehistoric Natives buried in mounds from eastern Uruguay. This last finding is particularly important to understand Uruguayan prehistory and history, raising the debate about who the mound builders were, and showing continuity between them, historic Charrúa Indians, and present populations.

On June 12th, 1833, The Times of London communicated: "Four savages, three men and one woman, belonging to a tribe of Indians called Charruas, which was almost entirely exterminated a short time back by the troops of the Republic at Uruguay […] have just been brought to Paris by M. Curel […]. Among them is the Cacique Vaimaca, surnamed Peru, who in 1814 entered the service of General Artigas…"; 169 years later, after being exposed in the Musée de l'Homme (Paris), the remains of the Cacique, named Vaimaca Perú or Pirú, returned to Uruguay. The process of repatriation was long, and the further destiny of the remains, despite detailed in a Law, as well as the possibility of studying them, were subject of debate.

Vaimaca was probably captured in April 1831 near Salsipuedes stream, in Northeastern Uruguay, during a massacre carried out against the Charrúa ethnic group[2]. "Salsipuedes" was the major event conducted by the Uruguayan government to exterminate this group, most of the men being killed, while women and children were captured and given to Spanish or "criollo" families. Soon after, François de Curel

obtained four captives from the government to be exposed in the Musée d´Histoire Naturelle, where they arrived in May 1933. The two oldest men, the sorcerer Senaqué and the chief Perú, died few months after the arrival, and soon, after having a baby girl, so did the woman; the youngest man escaped. When alive, the Indians were studied in the Museum, and after their death, also the two men. They are actually known as "the last Charrúa Indians" (Figure 1) after the title of P. Rivet´s detailed study[1].

In the 1980's decade, an Association of Native American descendants started a process to repatriate the Charrúa remains. In 2000, Uruguayan Parliament passed Law 17.256, which referred to the repatriation and inhumation of those remains in the National Pantheon. Finally, in July 18th, 2002, Vaimaca's skeleton, the only one preserved, was brought to Uruguay. The following day, a horse-drawn coach carrying the box with the remains covered by a Uruguayan flag went across the streets of Montevideo from the Airport to the Cemetery; a multitude greeted his way (Figure 2). Soon after, the Ministry of Education and Culture and the University of the Republic signed an agreement to study the remains, with some restrictions: the analysis should be done inside the Pantheon in five labour days, with the only exception of DNA analysis, to be performed at the University. The studies were encouraged by one Native-descents association, INDIA, while other interposed legal impediments that delayed the work. The Justice gave favourable verdict to continue our research, pointing out the importance of those scientific studies in the construction of National identity. However, in May 2003, the Parliament passed Law 17.767 that prohibited "experimentation and scientific studies" on the remains of Vaimaca Perú, thus preventing further studies.

At present, Uruguay is the only country in South America which lacks of Native ethnic groups and, despite advances in archaeological research, the knowledge of Uruguayan prehistory is still incomplete. When the Hispanic contact began, different groups populated the region, mainly related to the Macro-Charrúa ethnic group, as the Bohán, the Yaro, and the Guenoa/ Minuán; different historic sources describe their extermination. Another group, the Chaná, quickly disappeared, while the Guaraní entered the territory from the 15th until 19th century. With the exception of Guaraní, little is known about these groups, and even less about their physical or genetic characteristics. Then, the purpose of this study was to add as much information as

possible to the knowledge of historic Uruguayan hunter-gatherers, based on these unique Charrúa remains.

Vaimaca's identity was confirmed comparing morphological and pathological data with previous publications. According to different historic data, he was born ca. 1780 (which implies he was around 53 years old when he died, coherently with the state of cranial sutures, pubis, and vertebrae). He was very robust, and was 1.62 meters tall when alive; the same was estimated using a method based on Mexican Indians[3], which confirms its applicability in this case. His cranium was brachycrany (86.1), hypsicrany (77.5), tapeinocrany (89.9), euryprosopy (83.9), and contrarily to a previous determination, platyrhine (54.2). Based on metrics, Rivet[1] had proposed his affinity with R.Verneau's sub-brachycephalic Patagonic type; brachycrany is unusual in Uruguayan prehistoric individuals but at least two cases had been reported[4]. In our study, when compared to regional populations, cranial morphology showed most similarity in size and shape to remains from Argentina: Córdoba, followed by Santiago del Estero (both in central Argentina), while shape but not size was similar to Río Negro (Southern), San Blas and Paraná (Eastern), and also with Uruguay and southern Brazil (Santa Catarina preceramic) (Figure 3). The similitude with Córdoba can be supported by several historic sources that mentioned the presence of Charrúa Indians in Santa Fe, a neighbouring province. Moreover, the origin of the Charrúa Indians is discussed, and Bracco[5] indicates they were originally from the region between Paraná and Uruguay rivers, not far (less than 400 km) from Córdoba.

Peru's remains shows evidence of well-healed fractures in his left radius and 10th rib, consequence of a sabre wound apparently received in the Salsipuedes event, as well as a possible nasal fracture; another lesion in the right tibia was identified as periostitis without histopathological alterations, discarding a previous hypothesis proposing syphilis. After direct observation and X-Ray analyses, we also discard the existence of a bullet inside his cranium, as mentioned in a previous paper[10]. No other lesions are present with the exception of traces of osteoarthritis, being the body in excellent condition for his age. X-rays of tibias revealed two Harris lines, signal of a growth arrest at approximately 12 and 18 months old, that could be related to weaning. He had no caries and intermediate to high abrasion of the teeth (grades 3 to 5); shovel-shaped morphology was not seen, but other Native American characteristics were found, as the

prolongation of the enamel towards the roots and agenesis of the four third molars. The dentition was coherent with a diet based predominantly on meat, as concluded in other study based on the analysis of the enamel surface[11].

The quality of the DNA obtained limited the analyses to small fragments of mitochondrial DNA (mtDNA), but allowed to determine that it belongs to Amerindian haplogroup C. This haplogroup is one of the most frequent in Uruguayan Native-descent sub-population ([12] and references mentioned there), as well as in present and historic Native American groups from Southern South America[13,14], and in prehistoric mounds from Uruguay[15]. Besides the typical mutations characteristics of American haplogroup C (16223T, 16298C, 16325C, 16327T), the hypervariable region I (HVRI) also has 16051G that defines subhaplogroup C1d[16], and 16288C. This last, associated with the first five, was found in two Uruguayan prehistoric remains from a mound in Eastern Uruguay, the oldest radiocarbon dated in 1610 ± 90 years BP (AA81800), and in several contemporary Uruguayan individuals[12,17]. At present, no other sequences having these six mutations have been found outside Uruguay, and consequently we propose a local lineage, at least 1610 years old, being Vaimaca one of its carriers.

Although mtDNA only shows maternal heritage, its result is coherent with morphology: both can be related with populations from the Pampean region that extends from the Andes to the Atlantic Ocean and from Patagonia to Chaco, including Uruguay and part of Argentina and Brazil. Subhaplogroup C1d is distributed throughout the Americas, and also found in neighbouring regions, e.g. Kaingang from southern Brazil[18] and ancient remains from Arroyo Seco 2 in Argentina (GenBank Accession Numbers GU183399 and GU183400); possible relationships between Charrúas and these populations should be subject of a deeper analysis. Moreover, the presence of the rare mutation 16288C, shared by Vaimaca and prehistoric individuals buried in mounds, raises an old discussion related with the identity of the builders of those mounds that are distributed in eastern and northern Uruguay and southern Brazil. Different authors pointed out the Guenoas[9,19] , also called Minuanes, that conform the Macro-Charrúa ethnic group together with other minor groups. However, the similitude between the Charrúa chief and the mound-builders needs to be considered carefully: first, it is possible that individuals in the Macro-Charrúa group, defined culturally, share biological/molecular characteristics, and consequently, the builders could be ancestors

of Charrúas, Guenoas, or others from the same macro-group; and second, if practices involving exchange of spouses from different groups were common, then Vaimaca's maternal lineage could not reflect his ethnicity.

The last point to underline is the permanence of the maternal lineage through time, from around 1600 years ago to present day; this fact is contrary to the general belief and national identity based on the extinction of Native populations, showing the continuity of populations, at least in biological terms.

Methods

Morphological and pathological analysis: Sex was confirmed using qualitative[20] and quantitative methods, and age established[21,22]. Fifty-eight cranial measures and indexes[23] were calculated. Penrose's size and shape distances[5] were determined using eleven measures[6] chosen because its availability in the selected populations. Pathological conditions were registered and histological analysis of a lesion was performed. Several teeth characteristics were recorded; abrasion was evaluated using Molnar's scale[24]. X-rays of both maxillas were taken, as well as of several views of long bones and cranium. Stature was calculated using femur and tibia[3].

DNA analysis: Two DNA independent extractions from molars were made using a phenol-chloroform-silica method[25,26] in the ancient DNA Laboratory (LADNA, Humanities College, Uruguay); it has special conditions to avoid contamination[27]. RFLPs analyses were performed to identify the four common Amerindian mtDNA haplogroups using primers and conditions previously described[15,28] but using Platinum Taq (Gibco); sequencing of region 13232-13344 to confirm C haplogroup was performed in the Centro Técnico de Análisis Genéticos (CTAG, Uruguay) using ABI Prism 377 (Applied Biosystems, USA). HVRI region was replicated by polymerase chain reaction (PCR) using 5 set of primers previously described[28] and adding a new

set: L16035 (5'-TATTCTCTGTTCTTTCATGGG-3') - H16096 (5'-GTGGCGGCTAGTAATGRAC-3'). PCR fragments were inserted in pGem-T vectors transformed with a ligation mix of *E.coli* XL1 competent cells; transformed colonies were isolated in plaques of LB-agar containing ampiciline, IPTG and X-gal. Pre-cultures were prepared in LB and ampiciline, from which the plasmids were purified following a protocol of alkaline lysis. Plasmids with the insert, detected by electrophoresis, were sequenced (CTAG), using primers for promoters SP6 and T7 present in the vector, with BigDye Terminator kit (Applied Biosystems, USA). All sequences were corroborated by independent PCR reactions using the same set of primers (at LADNA), and sequenced in Macrogen Inc.(Korea), showing identical results.

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Author Contributions: M.S. conducted the research, analyzed DNA sequences, and wrote the main part of the paper. G.F. extracted DNA and replicated DNA fragments.

G.B. coordinates and made part of the pathological studies. R.M. analyzed pathologies, L.T. performed X-Rays; C.P. made histological analysis. R.O. performed teeth analyses; C.S. coordinated DNA fragments analyses. C.P., L.E. and C.S. purified, cloned and sequence DNA fragments. G.F. and P.C.H. made craniometric data analysis. I.B. registered morphology. P.C.H. and G. F. revised general aspects.

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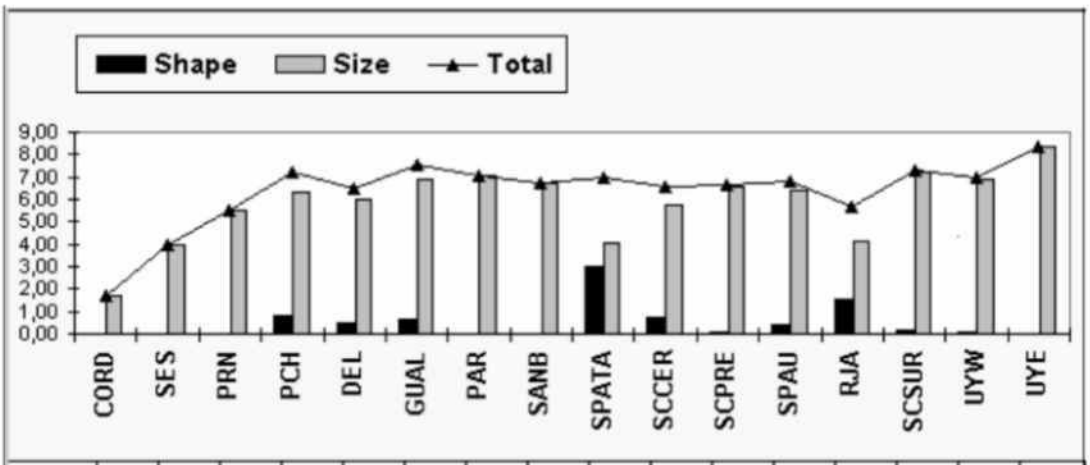
Legends:

Figure 1. **The last Charrua Indians.** Monument, by Edmundo Prati, Gervasio Furest Muñoz y Enrique Lussich, at Prado Park, Montevideo, Uruguay, based on drawings made in Paris, France (photo by MS). Vaimaca Perú is stand up in the back.

Figure 2. **Vaimaca Perú's remains entering the Central Cemetery** in their way to the National Pantheon, Montevideo, Uruguay, 19th July 2002. (Photo courtesy of Ruben Belrame).

Figure 3. **Penrose´s shape and size distances**[5]. Eleven common craniometric variables[6] were analyzed (1. Maximum cranial length, 2. Maximum cranial breadth, 3. Porion-bregma height, 4. Minimum frontal breadth, 5. Maximum frontal breadth, 6. Bizygomatic diameter, 7. Nasal height, 8. Nasal breadth, 9. Orbital breadth, 10. Orbital height, and 11. Interorbital breadth). The populations considered include published [6,7,8]

and unpublished data. Populations used: Chile: SATA (San Pedro de Atacama); Argentina: SES (Santiago del Estero); CORD (Cordoba), SANB (San Blas, Buenos Aires); DEL (Parana Delta), GUAL (Gualeguaychu, Entre Rios), PAR (Parana), PCH (Patagons from Chubut), PRN (Patagons from Rio Negro); Uruguay: UYE (Eastern Uruguay), UYW (Western Uruguay); Brazil (Sambaquis): RJA (Rio de Janeiro), SPAB (Sao Paulo), SCSUR (South of Santa Catarina), SCCER (Santa Catarina Ceramic), SCPRE (Santa Catarina Pre Ceramic).



shape	1.73	3.99	5.45	6.35	6.03	6.92	7.07	6.69	4.02	5.80	6.57	4.13	6.40	7.20	6.90	8.31
size	0.01	0.00	0.02	0.83	0.47	0.65	0.02	0.02	2.99	0.74	0.12	1.56	0.41	0.13	0.08	0.00
total	1.74	3.99	5.52	7.18	6.50	7.57	7.08	6.71	7.01	6.53	6.69	5.69	6.82	7.34	6.97	8.31