



DANA FIALOVÁ, KRISTÝNA BRZOBOHATÁ, EVA DROZDOVÁ, RADIM SKOUPÝ,
EVA CHOCHOLOVÁ, VLADISLAV KRZYŽÁNEK, IVO SVĚTLÍK, DOLORES DELGADO
MIRANDA, MERCEDES GONZÁLEZ FERNÁNDEZ, ARMANDO RANGEL RIVERO,
ANNA-MARIA BEGEROCK

NEW INSIGHTS TO REVEAL THE POSSIBLE GUANCHE ORIGIN OF THE CUBAN MUMMY OF THE "PERUVIAN MINER"

ABSTRACT: *The Montané Anthropological Museum of the University of Havana in Cuba houses a male mummy in its collection, which is called the "Peruvian Miner". During the 1970s, the origin of this mummy was determined to be Peruvian. The supine body position of the mummy was believed to be the result of an accident in a "Peruvian Mine", one that prevented a burial in a squat position, which was typical for pre-Columbian cultures. Recent research in 2015 has shown that this male individual is not in a squat position. A macroscopic investigation, especially observing most of all the positioning of the body, but also the type of mummification of the body and its preservation did suggest a possible origin from the pre-Hispanic inhabitants of Tenerife, (Canary Islands, today Spain), the Guanche. Three approaches were used to verify or refute the South American origin of the mummy and approve the hypothesis that it might be a Guanche. The first approach was to rule out the possibility that he was a miner. Therefore, elemental non-destructive analysis was performed by scanning electron microscopy with an energy-dispersive X-ray detector (SEM-EDX) to reveal possible contamination by metals mined in Latin America. The second approach was a genetic analysis. Mitochondrial ancient DNA (mtDNA) is more resistant to degradation than nuclear DNA in skeletal remains, due to the high number of mtDNA in the cell and its circular character. The poor preservation of DNA in the mummies was the reason for studying the whole mitogenome. This type of molecule has matrilineal inheritance, so it was used to trace the origin of the mummy. The third approach was radiocarbon dating to confirm or discard the pre-Columbian origin of the mummy.*

KEY WORDS: *Mummy of "Peruvian Miner" - Museo Montané - Cuba - aDNA - mtDNA - Electron microscopy - Radiocarbon dating - SEM-EDX detector*

Received 13 October 2023; Received in revised form 12 February 2024; Accepted 28 February 2024. Available online 7 May 2024.

© 2024 Moravian Museum, Anthropos Institute, Brno. All rights reserved.

DOI: <https://doi.org/10.26720/anthro.24.03.27.1>

INTRODUCTION AND AIM

The Montané Anthropological Museum of the University of Havana in Cuba houses a male mummy (naked body) in its collection, which is called the "Peruvian Miner". However, nothing was known about the man: not to which culture he once belonged, nor when he had died and got mummified nor where. Cuba itself had no cultures that mummified their ancestors. Also, nothing was known since he was in Cuba, therefore he remained quite mysterious.

The 1970s were the peak for Cuban archaeology in relation to mummy investigations. Cuban archaeologists did participate in excavations in Peru, and the two mummies they brought home were publicly unwrapped in the presence of Peruvian and Cuban scientists. These mummies are now in Museums in Holguín and Santiago de Cuba. Following the enthusiasm for pre-Columbian cultures of Peru, in Cuba Peruvian exhibitions were created to inform lay people of those treasures of the past and mummies already in Cuba were investigated. Among these mummies was one already in the Montané Anthropological Museum, which is the subject of the present manuscript and whose origins we are trying to uncover here.

The Montané Anthropological Museum is an Institution currently belonging to the Faculty of Biology of the University of Havana. It is named after its founder Don Luis Montané Dardé, an eminent Cuban researcher, who created it in 1903. It exhibits archaeological pieces of several pre-Hispanic cultures that populated the Caribbean area.

Unlike the Peruvian mummies well investigated and known in that time, this male individual is not in a squatted position, but lies on its back, the head slightly tilted towards his right shoulder, and arms stretched alongside the body and the hands on his upper thighs. Therefore, the researchers tried to find an explanation for the unusual burial position, then suspected pre-Columbian. Keeping the idea that this individual came also from Peru, they assumed an accident to have prevented a "proper" burial, which – so they did conclude – could have occurred in a pre-Columbian mine. Thus, the mummy received the name it still bears today: Peruvian Miner.

In 2015, in a special agreement between the CNPC (*Consejo Nacional del Patrimonio Cultural de la República de Cuba*) and the IECIM (*Instituto de Estudios Científicos en Momias, Madrid, Spain*), the Cuban Mummy Project was created, in order to investigate all mummies that are in Cuba. This international and interdisciplinary

research is not only focussing on the preventative conservation of the mummies in this country, but also investigating their cultural provenance and acquisition history. The case of the Peruvian Miner is especially interesting.

A macroscopic investigation, especially observing the mummification of the body, its preservation and most of all the positioning of the body did suggest a possible origin from the pre-Hispanic inhabitants of Tenerife, Canary Islands, today Spain, the Guanche.

Searching for historical documents on the shipment of (mummified) Guanche human remains from the Canary Islands and their arrival in Havana, one source (García González 2018) mentioned that pre-Hispanic human remains of Canary origin were exhibited in the Havana office of a certain doctor Miguel Gordillo Almeyda. These human remains were exposed in the second half of the 19th century, since the doctor died in 1898. He was being of Canary origin himself, practiced in the Cuban capital. Like many doctors at that time, the late 19th/ early 20th century, he showed a special interest in anthropological materials and started his own collection (García González 2008). Specifically, the skulls of this private collection were studied by Cuban anthropologists like Joaquín L. Dueñas y Pinto (1859–1910), Luis Montané (1849–1936) and Carlos de la Torre (1858–1950), who published their investigations (Montané 1885, Torres 1885). And in the first Regional Medical Congress of the Island of Cuba, in January 1890, the characteristics of the biological material of a Guanche mummy from Guía de Tenerife was debated (Anonym 1890). Searching further, notes of a historian from Tenerife, José Álvarez Rixo, even might mention the place of discovery, selling price, and ship for these human remains: in the Barranco de Ajabo in Villa de Adeje (Tenerife, Canary Islands) at the end of the year 1876 or 77 in which a walled-up cave was found containing a male mummy in a very good state of preservation. The mummy was found by peasants, who sold it for four ounces of gold. Then he was shipped to Havana on the Trinidad frigate that left from Santa Cruz de Tenerife in January 1878 towards the Cuban capital (Tejera Gaspar 1990).

However, since there is no documentation at the Montané Anthropological Museum in Havana, the origin of "Peruvian Miner" was still unproven. Scanning electron microscopy, radiocarbon dating, and the aDNA study of the body are therefore essential parts for determining the origin of the mummy.

These three approaches were used to verify or refute the South American origin of the mummy and approve

the hypothesis that it might be a Guanche. The methods used ranged from the least destructive to the most destructive. The first approach was to rule out the possibility that he was a miner. Therefore, elemental non-destructive analysis was performed by scanning electron microscopy with an energy-dispersive X-ray detector (SEM-EDX) on the outside and inside of the tooth sample to reveal possible contamination by metals mined in Latin America. The second approach was a genetic analysis. Mitochondrial ancient DNA (mtDNA) is more resistant to degradation than nuclear DNA in skeletal remains, due to the high number of mtDNA in the cell and its circular character. The poor preservation of DNA in the mummies was the reason for studying the whole mitogenome. This type of molecule has matrilineal inheritance, so it was used to trace the origin of the mummy. The unique approach used for next-generation sequencing was with a single-end kit, which reads DNA fragments from both reverse and forward strands and maximises the amount of information that is possible to obtain. The third approach was radiocarbon dating to confirm or discard the pre-Columbian origin of the mummy.

MATERIALS AND METHODS

a. Ancient DNA facility

The Laboratory of Biological and Molecular Anthropology (LBMA) of Masaryk University (Brno, Czechia) follows strict anti-contamination rules given

the nature of aDNA (Fulton *et al.* 2019, Knapp *et al.* 2012). Thus, all pre-PCR steps were performed in this special facility. Mitochondrial DNA profiles of LBMA staff were available to exclude potential contamination.

b. DNA extraction

Two types of tissue were taken by the team of the Montané Museum for aDNA extraction, dental and osseous. Both, the tooth and bone, showed good preservation. The bone sample was taken from the left femur, and it was hard with a shiny surface, without visible abrasion. The third upper right molar broke during extraction (*Figure 1*). The tissue samples were thoroughly washed with bleach (Savo®) and ultrapure water and exposed to UV light for 20 minutes ($\lambda 264$ nm). Before pulverization, the samples were cooled in a deep freezer at -80°C . After that, the samples were ground in an oscillating ball mill for one minute. The pulverized tissue was divided in total into 5 tubes (each of $100\text{ mg} \pm 10\%$) and placed in a lysis solution based on the publications by Juras *et al.* (2014) and Svensson *et al.* (2007). The mixture was composed of $900\text{ }\mu\text{L}$ EDTA (0.5 M , pH 8, Sigma-Aldrich), $50\text{ }\mu\text{L}$ urea (8.0 M , Sigma-Aldrich) and $50\text{ }\mu\text{L}$ proteinase K (20 mg/mL , Qiagen). Isolation was performed with MinElute® silicate columns (Qiagen) according to the modified protocol published by Yang *et al.* 1998 and Anderung *et al.* (2008) with negative blanks. DNA was eluted in $100\text{ }\mu\text{L}$ of elution buffer. Tubes with isolated DNA after confirmation of the presence of mitochondrial DNA were concentrated together in the next step.



FIGURE 1: Two types of tissue were available for aDNA extraction: the third upper right molar and the femur, the photo on the left is the "Peruvian Miner".

c. Amplification for confirmation of the presence of mtDNA

The HVRI and HVRII mtDNA sequences in the position range (16128–16348 and 45–287) were amplified by PCR in the Proflex® thermocycler with primers published in 2010 (Nilsson *et al.*). The master mix (total 22 µl) contained 13 µl KAPA2G Robust HotStart ReadyMix Kit (Roche), 1 µl primer F (0.5 µM), 1 µl primer R (0.5 µM); 2 µl BSA (1 mg/ml, Thermofisher) and 5 µl DNA. The cycling conditions were: initial denaturation 95 °C, 3 minutes; 40 cycles: 95 °C, 15 seconds, 60 °C, 15 seconds; 72 °C, 60 seconds and final extension 72 °C 10 minutes.

The results were visualized on 3% agarose (Agarose, low melting temperature, Sigma-Aldrich) stained with Nancy-520 (Sigma-Aldrich). The PCR products were purified with ExoSAP-IT™ Express PCR Product Cleanup Reagent (Thermofisher) and then with the MinElute® PCR kit. The purified products were mixed with sequencing primers (5 µl of 5 µM primer solution) and sent to commercial laboratories SEQme Company to Sanger sequencing. Samples with confirmed mtDNA (in total five) were concentrated prior to next-generation sequencing using an Amicon® Ultra-0.5 centrifugal filter (Millipore, Merck) at a final volume of approximately 20 µl to maximise the amount of template DNA.

d. Library preparation and mtDNA capture

The complete mitogenome library preparation was provided using ACCEL-NGS® 1S PLUS DNA LIBRARY KIT (Swift Biosciences), which performs single-stranded libraries. For indexing was used the 1S Plus Set A Indexing Kit (Cat. No. 16024) included in the kit with Illumina Truseq® LT adapters. Due to the fragmented nature of aDNA, the fragmentation step was excluded in the enzymatic preparation. For clean-up steps, magnetic SPRIselect™ beads (Beckman Coulter) were used. For observation of the amplification in real-time, we added 1 µl EvaGreen® (Biotium) to the master mix for indexing PCR. For this amplification, TOptical Thermocycler® (Biometra) was used, the same process was validated before in the study Šenovská *et al.* 2021. The sample was removed in the 15th cycle. The concentration was measured with a Quantus™ Fluorometer by Promega Corporation (Quantus) with a result of 26.3 ng/µl. The length of the fragments of the libraries was evaluated with a Fragment Analyzer™ (Advanced Analytical) in combination with the DNF 474 High Sensitivity NGS Fragment Analysis Kit (Figure 2) with the highest peak at 297 bp with adaptor and sequencing primers. The volume of DNA input to the pool was 100 ng (DNA was in an equimolar dose with other samples in the run).

The indexed library was enriched with the capture kit myBaits Expert Mito (Arbor Biosciences). The

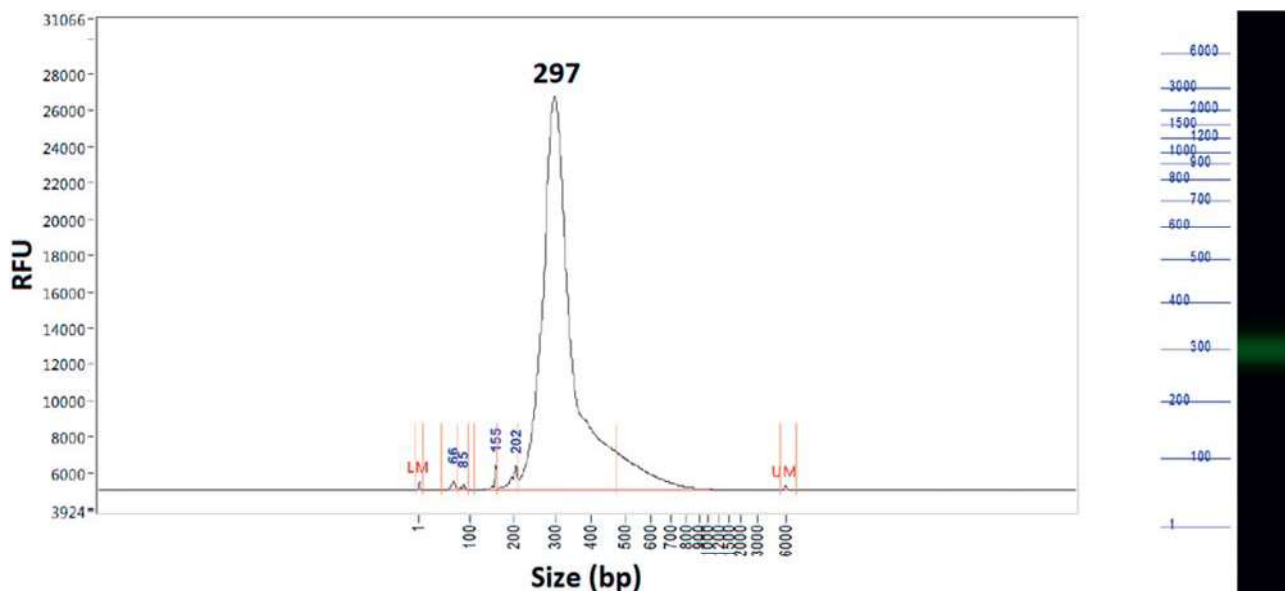


FIGURE 2: The length of the fragments of the libraries was evaluated with a Fragment Analyzer™ (Advanced Analytical) in combination with the DNF 474 High Sensitivity NGS Fragment Analysis Kit. The highest peak was 297 bp long.

hybridization process took 48 hours set at 60 °C using a manual (myBaits Manual v4.01) where the manufacturer's recommendations for ancient DNA were followed.

For the last amplification step, 19 (respectively, 21) cycles were chosen. The concentrations of the enriched libraries were then measured by the Quantus™ fluorometer from Promega Corporation (Quantus). The length of the library fragments was measured on 2200 TapeStation by Agilent Technologies (TapeStation) with the High Sensitivity D1000 ScreenTape Assay kit (Figure 3) with the highest peak at 273 bp. The final pool mean concentration was 20.1 ng/μl.

Next-generation sequencing and bioinformatic process

For next-generation sequencing, the Illumina platform NextSeq® 500 as 2 × 150 bp pair end with sequencing kit NextSeq® 500 v2 (Illumina) and positive control PhiX Control v3 (Illumina) was used. Sequencing was performed in the CEITEC MU laboratories of CF Genomics.

The sequencing data was first analysed in bcl2fastq Conversion Software v2.20 (Illumina). This step sorted the samples according to the barcoding indexes and cut off the adaptors and low-quality reads. Bioinformatic analysis was performed using Eager software v2.0.6 (Peltzer *et al.* 2016) where the MapDamage calculation could confirm the pattern of ancient DNA damage. In EAGER data were aligned to the mtDNA revised Cambridge Reference Sequence (rCRS, NC_012920.1)

(Andrews *et al.* 1999). The final consensus sequence was performed by Unipro UGENE v. 40.0 (Okonechnikov *et al.* 2012). For haplogrouping, MITOMASTER was used from the MITOMAP database (Mitomap 2019, Lott *et al.* 2013) and the EMPOP database (<http://empop.org>). The Haplocheck tool (Weissensteiner *et al.* 2021) was used for in-sample contamination detection (from the BAM file).

e. Radiocarbon dating

Radiocarbon dating was performed at the Department of Radiation Dosimetry, in the Nuclear Physics Institute of the Czech Academy of Sciences in Prague, and the HEKAL ATOMKI HAS laboratory in Debrecen, Hungary, using accelerator mass spectrometry (Kromer *et al.* 2013). For dating, a tooth sample was used. The activity of ¹⁴C and its combined uncertainty were expressed in years before present (BP) as the conventional radiocarbon dating after the Stuiver-Polach convention (Schneider *et al.* 1995). For the interpretation of the date of the ¹⁴C analysis results, the OxCal 4.4 calibration programme with radiocarbon calibration curve IntCal20 (Bronk, Lee 2013) was used. After fitting the uncertainties given by the radiocarbon calibration curve, the conventional radiocarbon age and its combined uncertainty were converted to the interval of the calibrated age.

f. SEM-EDX

For the determination of elemental composition, SEM-EDX analysis (scanning electron microscopy with

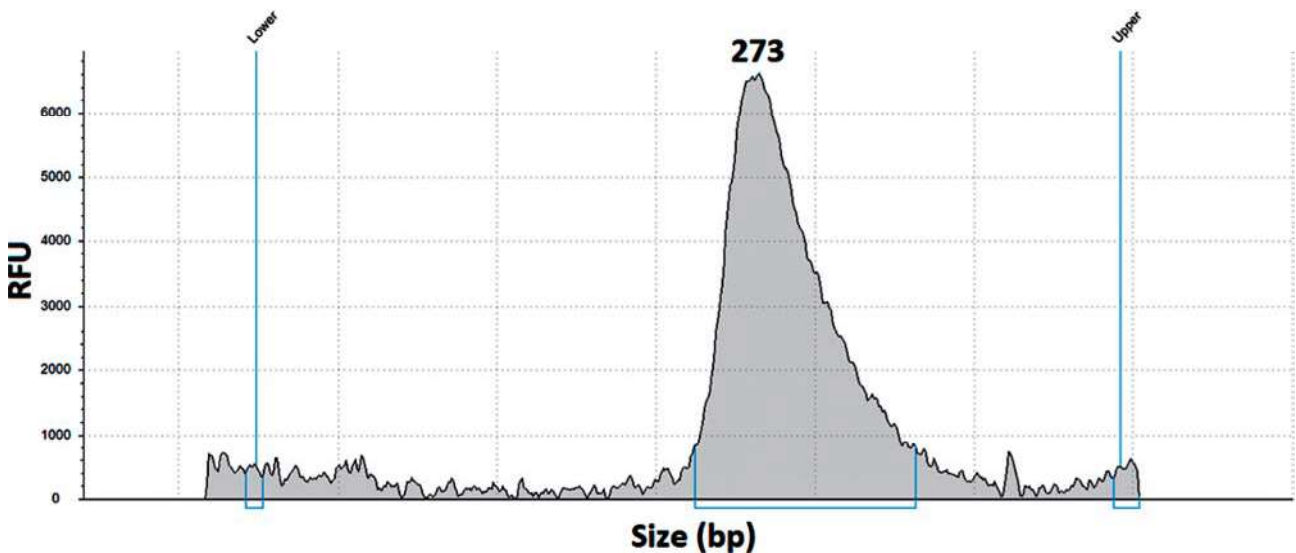


FIGURE 3: The length of the fragments of the libraries was measured on 2200 TapeStation by Agilent Technologies with the High Sensitivity D1000 ScreenTape Assay kit. The highest peak was 273 bp long.

TABLE 1: Polymorphisms detected in the mitogenome of the "Peruvian Miner". Diagnostic polymorphisms for the H1 haplogroup are in bold.

Position	Revised Cambridge reference (coverage in sample)	Variant in sample (coverage in sample)	GenBank full-length sequences (%)
Diagnostic polymorphisms for H1 haplogroup			
263	A	G (2)	95.034
750	A	G/A (10/1)	98.298
1438	A	G (13)	94.912
3010	G	A/G (11/1)	14.303
4769	A	G (13)	97.623
8860	A	G (16)	98.491
15326	A	G (11)	98.655
16519	T	C/T (2/2)	62.886
Extra polymorphisms			
73	A	G/A (4/1)	76.243
12612	A	G/A (10/8)	5.050
12904	A	T/A (11/6)	0
14766	C	T/C (7/4)	77.121
15452	C	A/C (7/3)	9.230
16126	T	C/T (6/2)	11.170
Extra polymorphisms with low coverage (possible postmortem damage)			
3106	C	T (1)	0
3554	T	A/T (1/1)	0
3638	T	C/T (1/1)	0
8602	T	C/T (5/4)	0.156
9598	T	C/T (3/2)	0

energy-dispersive X-ray spectroscopy) was used. It was performed by a Magellan 400 SEM (FEI) equipped with an Octane Elect Super EDS (EDAX). All measurements were taken at an acceleration voltage of 30 kV. The sample was mounted on aluminium stubs using double-sided carbon tape, and it was observed without any surface modification (no coating or plasma cleaning). The EDX spectrum was performed on the exterior side of the tooth and the EDX mapping was performed on the dental enamel incision.

RESULTS

Mitochondrial DNA

Single-end indexing preparation was performed. Thus, EAGER provided results for single separated

strands. In R1 0.636% of endogenous DNA was detected and in R2 0.550%. Coverage $> 1\times$ in R1 was 97.28% and in R2 99.07%. The mean coverage in R1 was 6.1654 and R2 5.6271. The result in BAM files from R1 and R2 was merged by Unipro UGENE v. 40.0 (Okonechnikov *et al.* 2012). After that, Haplocheck (Weissensteiner *et al.* 2021) was used for in-sample contamination detection (from the BAM file), and no contamination was detected. Thus, the mean coverage of the merged file was calculated on 9. In total nineteen variants (*Table 1*) in mitochondrial DNA were found in the sample of the "Peruvian Miner". Most variants are common according to GenBank (Benson *et al.* 2013). In *Table 1* are also visible some rare or unique mutations.

Mitomaster uses HaploGrep2 (Weissensteiner *et al.* 2016) and EMPOP uses EMMA (Röck *et al.* 2013) both with Phylotree 17 (van Oven, Kayser 2009) for haplogroup

TABLE 2: Results of the radiocarbon dating of collagen in the tooth of the mummy of "Peruvian Miner". Tooth sample quality rating: 1 – best, 6 – unsatisfactory. For samples with a quality rating of 4.5 or worse, more careful handling of dating results is recommended. P – total probability. In bold, the intervals include periods of probable tooth eruption. ** combined interval, in detail in Figure 4.

Laboratory Number of Sample	Sample characteristics	Concentration of Collagen (mg/g)	Conventional Radiocarbon Age (Years BP)	Calibrated Age, Main Interval (Years AD)	P [%]
22_0034	Tooth, hard, quality 1.5	175	967 ± 14	1027 – 1154	95.4**

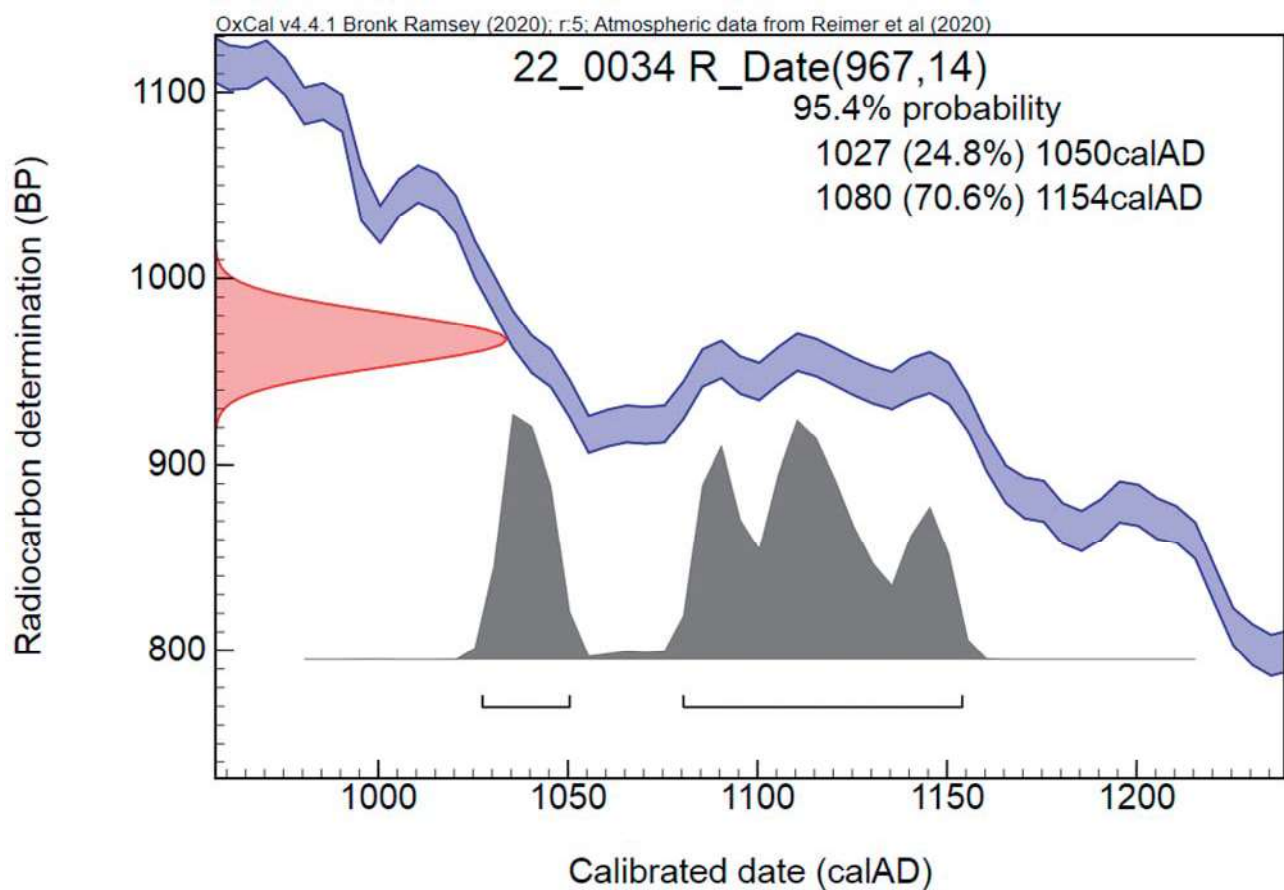


FIGURE 4: Calibration of the results of the ^{14}C analysis: ^{14}C activity, expressed in years of the Convention Radiocarbon Age on the vertical axis; calibration curve IntCal20, double line in the diagram; curves of the probability density of the sample origin in a given year, black areas on the horizontal axis; resulting time intervals (calibrated age) corresponding to the probability density below the horizontal line for total 95.4% probability.

determination. The predicted haplogroup is H1. The H1 haplogroup is predicted according to these diagnostic polymorphisms: 16519Y 263G 315.1C 750G 1438G 3010A 4769G 8860G 15326G. There is one missing mutation in sample 315.1C, but this region was not covered.

However, the obtained polymorphisms can be divided into three categories (*Table 1*). The first category is the group of diagnostic polymorphisms mentioned. The second and third categories do not have SNPs defining haplogroup determination. A second category

is a group of extra polymorphisms that have high coverage, so we consider them authentic. A third category is a group of polymorphisms that are extra with low coverage and unique occurrence in populations (according to GenBank), so this category could be interpreted as *postmortem* mutations. This applies to five mutations: 3554A 3638C 3106T 8602C 9598C. In other polymorphisms, there is visible *postmortem* damage, also. During diagenesis, the most common *postmortem* mutations are due to hydrolytic and oxidative processes. In mummified tissue, oxidation will be the main process of aDNA damage. This causes mainly transitions (e.g. C > U), but substitution (A > G and T > C) is common, too (Gilbert *et al.* 2003).

An extra polymorphism from the second category is interesting SNP 12904T (covered in the sample by 11 times) which seems to be an individual mutation of the "Peruvian Miner", not occurring in the world population (0% in GenBank).

Radiocarbon dating

The results of radiocarbon dating are reported in Table 2 (for an uncertainty interval of 2σ), which

corresponds to a probability of approximately 95%. The total absolute probability rate P in Table 2 mentioning the calibrated age interval was based on the expanded combined uncertainty of ^{14}C (2σ) and was calculated by the calibration programme. The age of isolated collagen should approximately correspond to the time of eruption of the analysed tooth of the individual (Handlos *et al.* 2018, Bárta, Štolc 2007). Radiocarbon dating confirmed the pre-Columbian origin of the sample. The main interval in calibrated age is between 1027 and 1154 AD with a probability of 95.4%. Figure 4 is the calibration diagram for the sample. The probability density curve is associated with the horizontal axis of the plot. The calibration age intervals for the 95.4% probability level are detailed. For the tooth sample, these are absolute probabilities.

SEM-EDX

The EDX spectrum of the exterior side of the dental enamel in Figure 5 and the EDX map of the dental enamel incision shows that there is no contamination of external chemical elements except aluminium (which was packaging material) and silicon from the soil. Figure

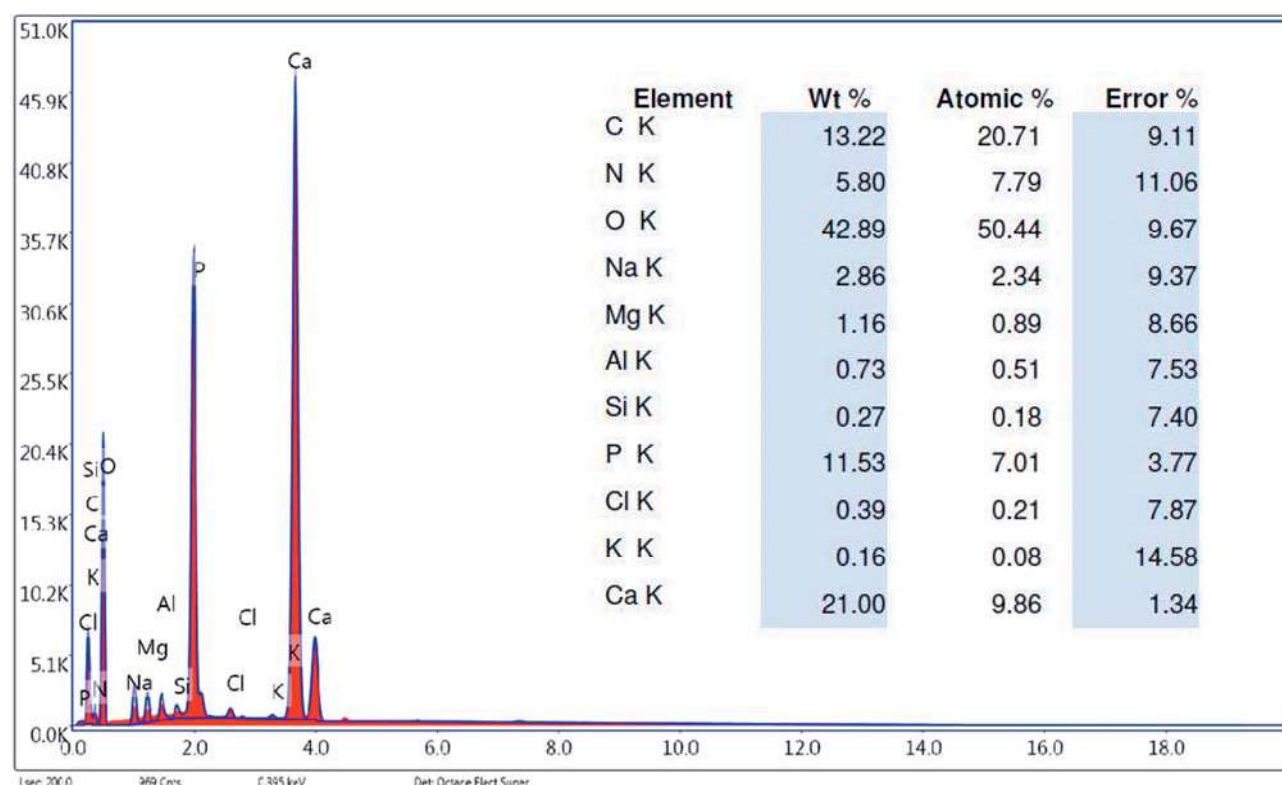


FIGURE 5: EDX spectrum of the external side of dental enamel.

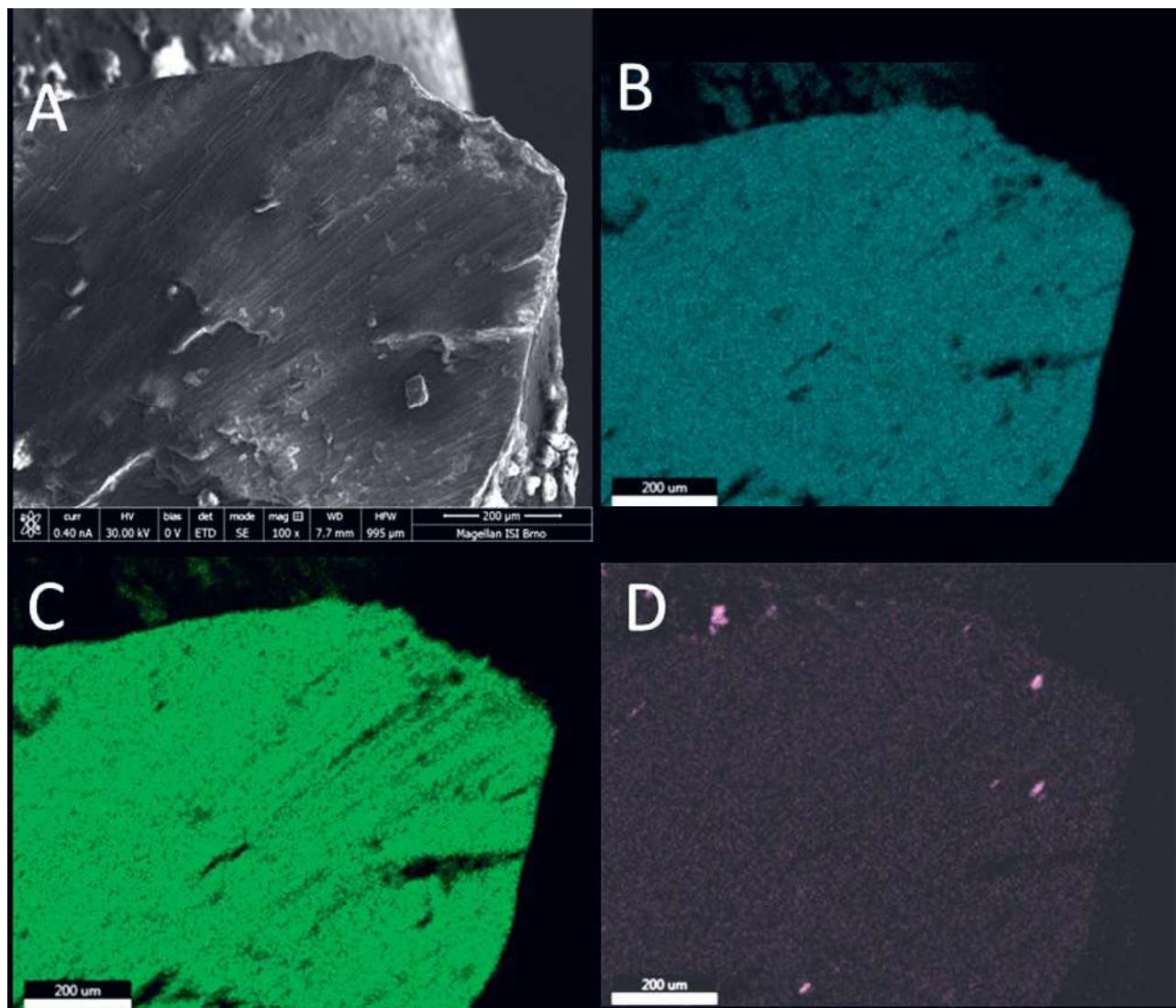


FIGURE 6: Tooth surface of one sample in different scanning modes A) SEM image of dental enamel incision; B) EDX map of phosphorus (dental enamel); C) EDX map of calcium (dental enamel); D) EDX map of silicon (soil contamination see violet areas).

6A is a secondary electron micrograph of a dental enamel incision. *Figure 6B* (phosphorus map) and *Figure 6C* (calcium map) show typical chemical elements of dental enamel. *Figure 6D* is a map of silicon that is typically present on the sample surface coming from the soil without any gradient inside.

After that, we performed EDX spectra of the dental root, dental calculus, and suspected debris on the external side. But also in these other places, no special (contaminating) elements were found. The suspected debris (*Figure 7*) was identified by EDX as organic due

to the dominant amount of carbon and other biogenic elements.

DISCUSSION

The macroscopic investigation of the mummy did indicate a Guanche origin of the male body on display in the Montané Anthropological Museum of the University of Havana, Cuba. Historical hints in documents in archives in Havana and Tenerife did

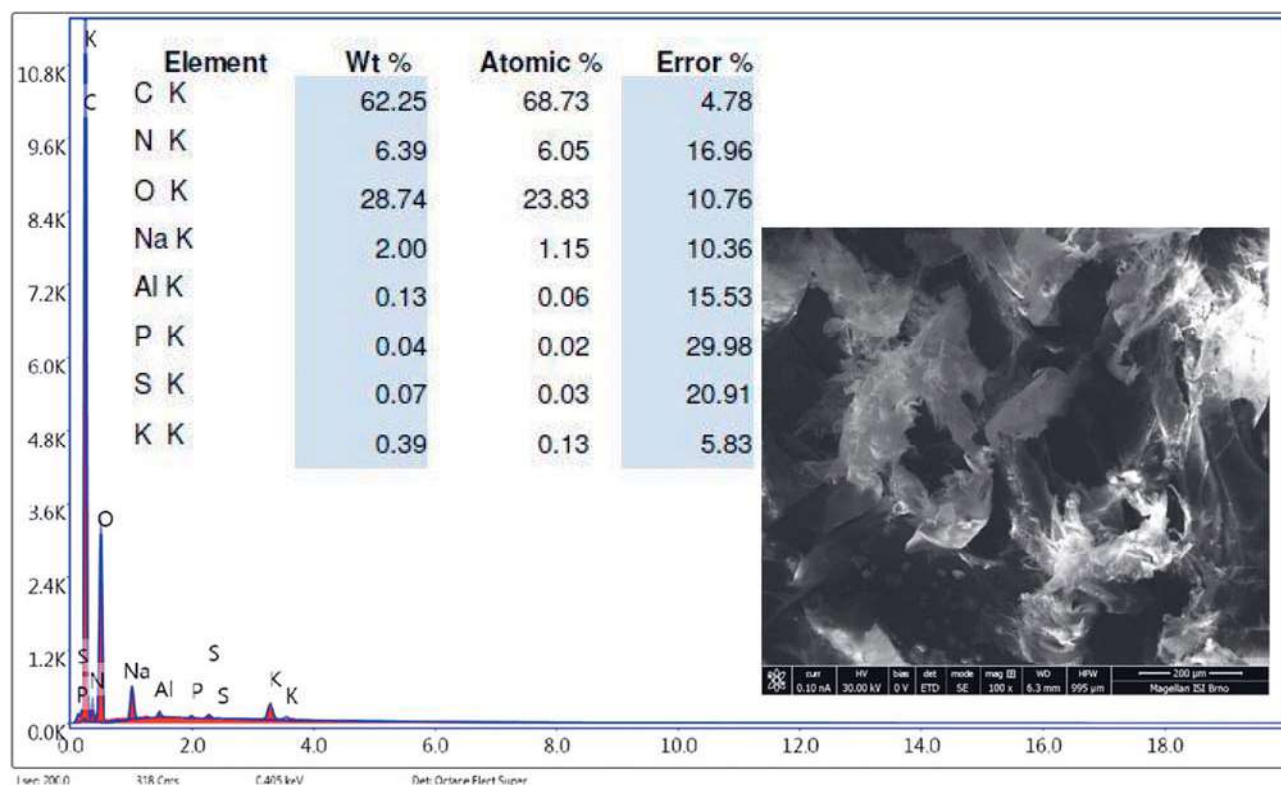


FIGURE 7: EDX spectrum and SEM image of organic debris on the external side of the tooth.

suggest that this mummy might have been exported in the 19th century, from a cave in Tenerife, to be displayed in the Cuban capital and to be studied there, too. But as no documentation is preserved in the museum it is in today, its origin was forgotten, declared pre-Columbian in the 1970s and supposed to be Guanche from 2017. Furthermore, the original burial place, supposedly a mine, needed to be confirmed or ruled out. Therefore, a genetic analysis was necessary to confirm the origin of the mummy.

The SEM-EDX analysis did not confirm the presence of any contaminating elements that could come from a mine. Even metals used in ancient Peru (Petersen, Brooks 2010) such as gold, silver, copper, tin, lead or platinum have not been found. More precise chemical analytic destructive methods would have to be used to identify elements within the detection limit of EDX. But the suitability of the SEM-EDX analysis was proven in several articles in which human habits through dental calculus were studied (Fialová *et al.* 2017, Charlier *et al.* 2018, Radini *et al.* 2019). Therefore, we assume that the individual could not have stayed in the mine either during life or after death. We are convinced he was not

"the miner". The big advantage of this analysis is non-destructiveness, which is appropriate concerning the following molecular and dating methods.

Radiocarbon dating confirmed the pre-Hispanic origin of the mummy, specifically between 1027 and 1154 AD with a probability of 95.4%. This dating corresponds to many mummified remains analysed from various areas of the island of Tenerife (Anaga, El Chorrillo, Araya, Igueste de San Andrés, Malpaís de Candelaria, Barranco Pilon, Adeje), corresponding to the human remains of the Guia area in Tenerife of an age of between GX-18740: 1092 ±81 B.P. (Arco *et al.* 1997).

Remains analysed by the Canarian Institute of Paleopathology and Bioanthropology offer dates between the 9th century (885), and the 14th (1355, Adeje). The age of the best-preserved Guanche mummy conserved in the MAN (National Archaeological Museum) of Madrid is fixed between 1154 and 1260 AD, with at least one 96.3% probability (Sánchez, Gómez 2018).

Genetic analysis was successfully sequenced using a unique next-generation sequencing approach with a single-end kit with 99.07% coverage for the reverse

strand despite the extensive *postmortem* damage. Haplogroup H1 was determined, which considering his pre-Columbian age confirmed the assumption that the mummy of the "Peruvian Miner" cannot be Native American, which has haplogroups A, B, C and D (Merriwether *et al.* 1995, Bonatto *et al.* 1997, Fuselli *et al.* 2003, Rodríguez-Delfín *et al.* 2001). The H1 haplogroup is typical of European origin with a frequency range between 40% and 50%. According to Roostalu *et al.* (2006), H1 formed around 22,500 years ago. In the database of ancient mitogenomes AmtDB (Ehler *et al.* 2019), we found only 4 individuals with haplogroup H1 from the 12th century who were from Spain (Olalde *et al.* 2019). This haplogroup was confirmed in many populations of Guanches (Rodríguez-Varela *et al.* 2017, Rando *et al.* 1999, Fregel *et al.* 2019). Due to the above evidence, we assume that the mummy of the "Peruvian Miner" is not a miner, nor is he from Peru, and could be Guanche.

CONCLUSION

Three approaches were used to rule out the possibility that the mummy of man is that of a "Peruvian Miner" – from pre-Columbian Peru. However, all studies carried out suggest that he might be Guanche. Firstly, an SEM-EDX analysis did show an absence of any contaminating (such as gold, silver, copper) elements on the surface and inside the tooth sample. The suggested burial of this man in a mine was proven to never have happened. This man never worked in a mine for a long time, nor was he buried in one. Secondly, radiocarbon dating confirmed the pre-Hispanic origin of the mummy (1027–1154 AD), which corresponds to many mummified remains of Guanches.

And finally, the genetic analysis of mitochondrial DNA confirmed that the matrilinear origin of the mummy with the H1 haplogroup cannot be a pre-Columbian Native American. But, since this haplogroup was detected in populations of Guanches as well as in Spain in the 12th century, the mummy man is most probably of Guanche origin. Only few mummies of this culture are preserved and known. Some can be found in Canary museums and even less in worldwide collections. The one in Havana was unknown outside of Cuba until most recently. As Guanche mummies are so rare and only few is known about Guanche burial rituals and mummification techniques, the find of this mummified body is very valuable for the scientific community. But more importantly, with this research

and its results we were able to reconstruct parts of the man's origin and history, thus re-individualizing this human being, that was decontextualized before.

ACKNOWLEDGEMENTS

Especially we are grateful to Dr. Eusebio Leal Spengler (†) who initiated the *Cuban Mummy Project*. This project is under the auspices of the *Cuban National Council for World Heritage*. We thank Gladys Collazo Usallán for her kind support. Also, we thank all researchers and contributors: for the Anthropological Museum Montané, Dr. Carlos Arredondo Antúnez and Dr. Dodany Machado Mendoza, from the Institute of Forensic Medicine (Havana, Cuba).

REFERENCES

- ANONYM 1890: *Primer Congreso Medico Regional de la Isla de Cuba celebrado en La Habana en enero de 1890*. A. Álvarez Y Compania. La Habana.
- ARCO M. C., ARCO M. M., ATIÉNZAR E., ATOCHE PEÑA P., MARTÍN M., RODRÍGUEZ C., ROSARIO C., 1997: Dataciones absolutas en la Prehistoria de Tenerife. In: A. Millares Cantero, M. Lobo Cabrera, P. Atoche Peña (Eds.): *Homenaje a Celso Martín de Guzman*. Pp. 67–77. Universidad de las Palmas de Gran Canaria, Servicio de Publicaciones.
- ATOCHE P., ARCO M. C., 2023: Carbono 14 y la colonización protohistórica de las Islas Canarias: la importancia del contexto arqueológico en la interpretación histórica. *Anuario de Estudios Atlánticos* 69: 1–34.
- ANDERUNG C., PERSSON P., BOUWMAN A., ELBURG R., GOTHERSTROM A., 2008: Fishing for ancient DNA. *Forensic Science International-Genetics* 2: 104–107. [online] DOI: 10.1016/j.fsigen.2007.09.004
- ANDREWS R. M., KUBACKA I., CHINNERY P. F., LIGHTOWLERS R. N., HOWELL N., 1999: Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nature Genetics* 23, 2: 147. DOI:10.1038/13779
- BÁRTA P., ŠTOLC JR. S., 2007: HBCO Correction: Its impact on archaeological absolute dating. *Radiocarbon* 49, 2: 465–472.
- BENSON D. A., CAVANAUGH M., CLARK K., KARSCH-MIZRACHI I., LIPMAN D. J., OSTELL J., SAYERS E. W., 2013: GenBank. *Nucleic Acids Research (Database issue)* 41: D36–42. DOI: 10.1093/nar/gks1195
- BONATTO S.-L., SALZANO M. F., 1997: Diversity and age of the four major mtDNA haplogroups, and their implications for the peopling of the New World. *American Journal of Human Genetics* 61: 1413–1423.
- BRONK R. C., LEE S., 2013: Recent and Planned Developments of the Program OxCal. *Radiocarbon* 55, 2–3: 720–730.

- CHARLIER P., WEIL R., RAINSARD P., POUPON J., BRISARD J. C., 2018: The remains of Adolf Hitler: A biomedical analysis and definitive identification. *European Journal of Internal Medicine* 54: 10–12.
- EHLE E., NOVOTNÝ J., JURAS A., CHYLEŇSKI M., MORAVČÍK O., PAČES J., 2019: AmtDB: a database of ancient human mitochondrial genomes. *Nucleic Acids Research* 47, (D1): D29–D32. DOI: 10.1093/nar/gky843
- de la TORRE Y HUERTA C., GONZÁLES Y CURQUEJO A., 1885: *La Enciclopedia. Revista mensual. Dirigida por los Dres. Propaganda Literaria, Habana.*
- FIALOVÁ D., SKOUPÝ R., DROZDOVÁ E., PATÁK A., PIŇOS J., ŠÍN L., BENUŠ R., KLÍMA B., 2017: The Application of Scanning Electron Microscopy with Energy-Dispersive X-Ray Spectroscopy (SEM-EDX) in Ancient Dental Calculus for the Reconstruction of Human Habits. *Microscopy and microanalysis* 23, 6: 1207–1213.
- FREGEL R., ORDÓÑEZ A. C., SANTANA-CABRERA J., CABRERA V. M., VELASCO-VÁSQUEZ J., ALBERTO V., MORENO-BENÍTEZ M. A., DELGADO-DARIAS T., RODRÍGUEZ-RODRÍGUEZ A., HERNÁNDEZ J. C., PAIS J., GONZÁLEZ-MONTELONGO R., LORENZO-SALAZAR J. M., FLORES C., CRUZ-de-MERCADAL M. C., ÁLVAREZ-RODRÍGUEZ N., SHAPIRO B., ARNAY M., BUSTAMANTE C. D., 2019: Mitogenomes illuminate the origin and migration patterns of the indigenous people of the Canary Islands. *PLoS One* 20, 14(3): e0209125. DOI: 10.1371/journal.pone.0209125
- FULTON T. L., SHAPIRO B., 2019: Setting Up an Ancient DNA Laboratory. In: B. Shapiro, A. Barlow, P. Heintzman, M. Hofreiter, J. Paijmans, A. Soares (Eds.): *Ancient DNA. Methods in Molecular Biology* 1963. Pp. 1–13. Humana Press, New York. DOI: https://doi.org/10.1007/978-1-4939-9176-1_1
- FUSELL S., TARAZONA-SANTOS E., DUPANLOUP I., SOTO A., LUISELLI D., PETTENER D., 2003: Mitochondrial DNA diversity in South America and the genetic history of Andean highlanders. *Molecular Biology and Evolution* 20, 10: 1682–1691. DOI: <https://doi.org/10.1093/molbev/msg188>
- GARCÍA GONZÁLES A., 2008: *The canary Miguel Gordillo in Cuban science from the 19th century.* Editorial IDEA, Tenerife.
- GARCÍA GONZÁLEZ A., 2018: Gordillo y Almeyda, Miguel. Santa María de Guía, Gran Canaria (Las Palmas), 28.III.1824 – La Habana (Cuba), 22.II.1898. Médico, poeta. *The Bibliographic Dictionary of the Royal Academy of History of Spain.* Real Academia de la Historia. Online <https://dbe.rah.es/biografias/79163/miguel-gordillo-y-almeyda>
- GILBERT M. T., WILLERSLEV E., HANSEN A. J., BARNES I., RUDBECK L., LYNNERUP N., COOPER A., 2002: Distribution patterns of postmortem damage in human mitochondrial DNA. *American Journal of Human Genetics* 72, 1: 32–47. DOI: 10.1086/345378
- GUPTA S. K., POLACH H. A., 1985: *Radiocarbon dating practises at ANU.* Australian National University, Canberra.
- HANDLOS P., SVĚTLÍK I., HORÁČKOVÁ L., FEJGL M., KOTÍK L., BRYCHOVÁ V., MENGISOVÁ N., MAREČKOVÁ K., 2018: Bomb peak: radiocarbon dating of skeletal remains in routine forensic medical practice. *Radiocarbon* 60, 4: 1017–1028.
- JURAS A., DABERT M., KUSHIAREVICH A., MALMSTRÖM H., RAGHAVAN M., KOSICKI J. Z., METSPALU E., WILLERSLEV E., PIONTEK J., 2014: Ancient DNA reveals matrilineal continuity in present-day Poland over the last two millennia. *PLoS One* 9: 1–9. DOI: <https://doi.org/10.1371/journal.pone.0110839>
- KNAPP M., CLARKE A. C., HORSBURGH K. A., MATISOO-SMITH E. A., 2012: Setting the stage – building and working in an ancient DNA laboratory. *Annals of Anatomy* 194, 1: 3–6. DOI: <http://dx.doi.org/10.1016/j.aanat.2011.03.008>
- LOTT M. T., LEIPZIG J. N., DERBENEVA O., XIE H. M., CHALKIA D., SARMADY M., PROCACCIO V., WALLACE D. C., 2013: mtDNA variation and analysis using MITOMAP and MITOMASTER. *Current Protocols in Bioinformatics* 1, 123: 1–26.
- MERRIWETHER D. A., ROTHHAMMER F., FERRELL R. E., 1995: Distribution of the four founding lineage haplotypes in Native Americans suggests a single wave of migration for the New World. *American Journal of Physical Anthropology* 98:411–430.
- MITOMAP, 2019: *A Human Mitochondrial Genome Database.* <http://www.mitomap.org>
- myBAITS MANUAL v4.01 <https://arborbiosci.com/wp-content/uploads/2018/04/myBaits-Manual-v4.pdf> (Accessed 1 August 2020).
- MONTANÉ L., 1885: *Un cráneo guanche. Estudio Craneológico.* Imprenta La propaganda literaria, La Habana.
- NILSSON M., POSSNERT G., EDLUND H., BUDOWLE B., KIELSTRÖM A., ALLEN M., 2010: Analysis of the Putative Remains of a European Patron Saint–St. Birgitta. *Plos one.* DOI: <https://doi.org/10.1371/journal.pone.0008986>
- OKONECHNIKOV K., GOLOSOVA O., FURSOV M., UGENE team, 2012: Unipro UGENE: a unified bioinformatics toolkit. *Bioinformatics* 28, 8: 1166–1167. DOI: 10.1093/bioinformatics/bts091
- OLALDE I., MALLICK S., PATTERSON N., ROHLAND N., VILLABA-MOUCO V., SILVA M., DULIAS C., EDWARDS C. J., GANDINI F., PALA M., SOARES P., FERRANDO-BERNAL M., ADAMSKIN B., BROOMANDKHOSHBAHT N., CHERONET O., CULLETON B., FERNANDES D., LAWSON A. M., MAH M., OPPENHEIMER J., STEWARDSON K., ZHANG Z., JIMÉNEZ ARENAS J. M., TORO MOYANO I. J., SALAZAR-GARCÍA D. C., CASTANYER P., SANTOS M., TREMOLEDA J., LOZANO M., GARCÍA BORJA P., FERNÁNDEZ-ERASO J., MUJICA-ALUSTIZA J. A., BARROSO C., BERMÚDEZ F. J., VIGUERA MÍNGUEZ E., BURCH J., COROMINA N., VIVÓ D., CEBRIÀ A., FULLOLA J. M., MAJÓ T., VERGÈS J. M., DÍAZ-CARVAJAL A., OLLICH-CASTANYER I., LÓPEZ-CACHERO F. J., SILVA A. M., ALONSO-FERNÁNDEZ C., DELIBES de CASTRO G., JIMÉNEZ ECHEVARRÍA J., MORENO-MÁRQUEZ A., BERLANGA G. P., RAMOS-GARCÍA P., RAMOS-MUÑOZ J., VIJANDE VILA E., AGUILELLA ARZO G., ESPARZA ARROYO Á., LILLIOS K. T., MACK J., VELASCO-VÁSQUEZ J., WATERMAN A., de LUGO

- ENRICH L. B., BENITO SÁNCHEZ M., AGUSTÍ B., CODINA F., de PRADO G., ESTALRRICH A., FERNÁNDEZ FLORES Á., FINLAYSON C., FINLAYSON G., FINLAYSON S., GILES-GUZMÁN F., ROSAS A., GONZÁLES V. B., GARCÍA ATIÉNZAR G., HERNÁNDEZ PÉREZ M. S., LLANOS A., CARRIÓN MARCO Y., COLLADO BENEYTO I., LÓPEZ-SERRANO D., SANZ TORMO M., VALERA A. C., BLASCO C., LIESAU C., RÍOS P., DAURA J., de PEDRO MICHÓ M. J., DIEZ-CASTILLO A. A., FLORES FERNÁNDEZ R., FARRÉ J. F., GARRIDO-PENA R., GONÇALVES V. S., GUERRA-DOCE E., HERRERO-CORRAL A. M., JUAN-CABANILLES J., LÓPEZ-REYES D., McCLURE S. B., MERINO PÉREZ M., FOIX A. O., SANZ BORRÀS M., SOUSA A. C., VIDAL ENCINAS J. M., KENNETT D. J., RICHARDS M. B., ALT K. W., HAAK W., PINHASI R., LALUEZA-FOX C., REICH D., 2019: The genomic history of the Iberian Peninsula over the past 8000 years. *Science* 363, 6432: 1230–1234. DOI: 10.1126/science.aav4040
- PARSON W., DÜR A., 2007: EMPOP—A forensic mtDNA database. *Forensic Science International Genetics* 1, 2: 88–92. DOI: 10.1016/j.fsigen.2007.01.018
- PETERSEN G. G., BROOKS W. E., 2010: *Mining and Metallurgy in Ancient Perú*. Special paper of Geological Society of America, Boulder. DOI: <https://doi.org/10.1130/SPE467>
- PELTZER A., JÄGER G., HERBIG A., SEITZ A., KNIPE C., KRAUSE J., NIESELT K., 2016: EAGER: efficient ancient genome reconstruction. *Genome Biology* 17: 60. DOI: 10.1186/s13059-016-0918-z
- RADINI A., TROMP M., BEACH A., TONG E., SPELLER C., McCORMIK M., DUDGEON J. V., COLLINS M. J., RÜHLI F., KRÖGER R., WARRINER C., 2019: Medieval women's early involvement in manuscript production suggested by lapis lazuli identification in dental calculus. *Science Advances* 5, 1. DOI: <https://doi.org/10.1126/sciadv.aau7126>
- RANDO J. C., CABRERA V. M., LARRUGA J. M., HERNÁNDEZ M., GONZÁLEZ A. M., PINTO F., BANDELT H. J., 1999: Phylogeographic patterns of mtDNA reflecting the colonization of the Canary Islands. *Annals of Human Genetics* 63, 5:413–428. DOI: 10.1046/j.1469-1809.1999.6350413.x
- RODRIGUEZ-DELFIN L. A., RUBIN-de-CELIS V. E., ZAGO M. A., 2001: Genetic diversity in an Andean population from Peru and regional migration patterns of Amerindians in South America: data from Y chromosome and mitochondrial DNA. *Human Heredity* 51, 1–2: 97–106.
- RODRÍGUEZ-VARELA R., GÜNTHER T., KRZEWIŃSKA M., STORÅ J., GILLINGWATER T. H., MacCALLUM M., ARSUAGA J. L., DOBNEY K., VALDIOSERA C., JAKOBSSON M., GÖTHERSTRÖM A., GIRDLAND-FLINK L., 2017: Genomic Analyses of Pre-European Conquest Human Remains from the Canary Islands Reveal Close Affinity to Modern North Africans. *Current Biology* 6, 27(21): 3396–3402. DOI: 10.1016/j.cub.2017.09.059
- RÖCK A. W., DÜR A., VAN OVEN M., PARSON W., 2013: Concept for estimating mitochondrial DNA haplogroups using a maximum likelihood approach (EMMA). *Forensic Science International Genetics* 7, 6: 601–609. DOI: 10.1016/j.fsigen.2013.07.005
- SÁNCHEZ CABRERO B., GÓMEZ ESPINOSA T., 2018: Datación mediante carbono 14 de la momia guanche del Museo Arqueológico Nacional. *Boletín del Museo Arqueológico Nacional* 37: 471–478.
- SVENSSON E. M., ANDERUNG C., BAUBLIENE J., PERSSON P., MALMSTRÖM H., SMITH C., VRETEMARK M., DDAUGNORA L., GÖTHERSTRÖM A., 2007: Tracing genetic change over time using nuclear SNPs in ancient and modern cattle. *Animal Genetics* 38, 4: 378–383. DOI: 10.1111/j.1365-2052.2007.01620.x
- ŠENOVSÁ A., DROZDOVÁ E., VACULÍK O., PARDY F., BRZOBOHATÁ K., FIALOVÁ D., ŠMERDA J., KOS P., 2021: Cost-effective straightforward method for captured whole mitogenome sequencing of ancient DNA. *Forensic Science International* 319: 110638. DOI: 10.1016/j.forsciint.2020.110638
- TEJERA GASPAS A., 1990: Apuntes sobre restos de los guanches encontrados en el siglo actual, de Jose Agustín Alvarez Rixo. *ERES Publicaciones del Organismo Autónomo de Museo Arqueológico y Etnográfico del Cabildo Insular de Tenerife. Serie de Arqueología* 1, 1: 121–134.
- de la TORRE Y HUERTA C., 1885: Cráneos Guanches. In: C. De la Torre y Huerta, A. Gonzáles y Curquejo (Eds.): *La Enciclopedia. Revista mensual Dirigida pro los Dres.* Pp. 196–201. Propaganda Literaria, Habana.
- van OVEN M., KAYSER M., 2009: Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Human Mutation Variation, Informatics and Disease* 30, 2: E386–E394. DOI: <http://www.phyloree.org>. doi:10.1002/humu.20921
- WEISSENSTEINER H., FORER L., FENDT L., KHERIKHAH A., SALAS A., KRONENBERG F., SCHOENHERR S., 2021: Contamination detection in sequencing studies using the mitochondrial phylogeny. *Genome Research* 31, 2: 309–316. DOI: 10.1101/gr.256545.119
- WEISSENSTEINER H., PACHER D., KLOSS-BRANDSTÄTTER A., FORER L., SPECHT G., BANDELT H. J., KRONENBERG F., SALAS A., SCHOENHERR S., 2016: HaploGrep 2: mitochondrial haplogroup classification in the era of high-throughput sequencing. *Nucleic Acids Research* 44, 1: 58–63. DOI: 10.1093/nar/gkw233
- YANG D. Y., ENG B., WAYE J. S., DUDAR J. C., SAUNDERS S. R., 1998: Improved DNA extraction from ancient bones using silica-based spin columns. *American Journal of Physical Anthropology* 105: 539–543.

Dana Fialová¹, Kristýna Brzobohatá¹,
Eva Drozdová^{1*}, Radim Skoupý²,
Eva Chocholová¹, Vladislav Krzyžánek²,
Ivo Světlík³, Dolores Delgado Miranda⁴,
Mercedes González Fernández⁴,
Armando Rangel Rivero⁵,
Anna-Maria Begerock⁴

¹Laboratory of Biological and Molecular
Anthropology, Department
of Experimental Biology, Faculty
of Science, Masaryk University, Brno,
Czech Republic;
E-mail: drozdova@sci.muni.cz

²Institute of Scientific Instruments of the
Czech Academy of Sciences, Brno,
Czech Republic

³CRL Czech Radiocarbon Laboratory,
joint workplace of Nuclear Physics
Institute and Institute of Archeology,
Prague, Academy of Science of the
Czech Republic

⁴IECIM (Institute for the Scientific
Study of Mummies), Madrid, Spain

⁵Montané Anthropological Museum,
of the University of Havana, Havana,
Cuba

* Corresponding author.