

## Article

# Maternal Lineages during the Roman Empire, in the Ancient City of Gadir (Cádiz, Spain): The Search for a Phoenician Identity

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**Abstract:** Phoenicians were probably the first eastern Mediterranean population to establish long-distance connections with the West, namely the Iberian Peninsula, from the final Bronze to the early Iron Age. For a long time, these colonies all over the Mediterranean Sea directly depended on an important city administration, Gadir, the most important metropolis in the Western Mediterranean. Modern archaeological excavations were discovered in Cadiz (Spain), the ancient city of Gadir, as well as possible Phoenician burial places. The purpose of the present work is the molecular study of 16 individuals, (V–IV millennium B.C, V A.D.) from several burial places found in Cadiz, attempting to disclose their maternal biogeographical ancestry. Furthermore, the determination of a possible biological link between two individuals found buried together was also an objective of this investigation. Of all the 16 analyzed individuals, eight of them produced positive results. Three main lineages were found: HV0, H and L3b. In general, the results support an Eastern origin for this set of individuals, reinforcing the theory of a Phoenician origin. Due to their historical period, in some cases, it was not possible to discard a Roman origin. Finally, the maternal kinship between two individuals found buried together was discarded.

**Keywords:** Phoenicians; Iberia Peninsula; maternal lineage; mtDNA sequencing; kinship

## 1. Introduction

The multidisciplinary genetic, anthropological and archaeological study of ancient samples constitutes the most direct way of knowing historical populations and their particular movements. The Iberian Peninsula has long been a unique part of the ancient world. Despite its distancing from the rest of Europe, it has been consistently a connection of trade, commerce and cultural relationships (Dietler and López-Ruiz 2009).

From the Bronze Age to the Renaissance, explorers and traders used the Peninsula as the transition between the Mediterranean and the rest of the world (Dietler and López-Ruiz 2009). Phoenicians were possibly the first eastern Mediterranean people to establish long-distance connections with the west, from the final Bronze to the early Iron Age. Their expansions and settlements increased in the south and Levant coast of the Iberia Peninsula, as well as in the Atlantic fringe (Tsirkin 1997; Arruda 2009; Groot 2012). With the Phoenicians, regional trade networks were dramatically enlarged resulting in an enormous economic, social and cultural development that changed not only the history of Iberia, but also had a great impact on the whole of Mediterranean history (Dietler and López-Ruiz 2009; Valério et al. 2012; Amadori et al. 2016).

Over the centuries, historians have tried to understand the Phoenicians' origins (Aubert 2001; Groot 2012; Padró 2012). Padró (2012) mentions that the Phoenicians were Canaanites, justifying this based on their language (Canaanite) and the fact that "Phoenicians" called themselves Canaanites (Aubert 2001; Groot 2012; Padró 2012). It is thought that both Hebrews and Phoenicians were descendants of the Proto-Canaanites (Padró 2012).

For a long time, Phoenician colonies all over the Mediterranean Sea directly depended on the Gadir administration, the most important metropolis in the Western Mediterranean (Padró 2012), located in the South of the Iberian Peninsula, which is now situated in the city of Cádiz. Recent archaeological expeditions discovered several burial places in this city that belong to the Roman Empire (I B.C, I A.D; and IV–V A.D centuries), which showed a lifestyle and burial characteristics similar to the Phoenicians. Nevertheless, this assumption should be taken with caution, since the presence of the Phoenicians in the Iberian Peninsula was shared with other populations, such as Tartessics, Punics, and Romans, so the human remains may or may not have a Phoenician origin.

Concerning the molecular analysis of the "Phoenician origin", there are few investigations dealing with their possible maternal ancestry. For instance, Zalloua et al. (2008) investigated the Phoenician male traces in more than a thousand samples, but in extant populations (Zalloua et al. 2008). On the other hand, in 2016, Matisoo-Smith et al. (2016) described for the first time the results of a molecular investigation on an ancient Phoenician sample. In their study, one individual was found in North Africa, and through the next-generation sequencing (NGS) technique, they were able to identify a European lineage by comparing it with modern populations (Matisoo-Smith et al. 2016).

From the molecular point of view, populations living geographically close and/or with recently shared ancestry will present similar haplotypes that are grouped by relatedness into genetic haplogroups (Cavalli-Sforza and Feldman 2003; Emery et al. 2015). Thus, the association between a geographical region and a mitochondrial DNA (mtDNA) provides the basis for using mtDNA haplogroups to deduce an individual's genetic maternal biogeographical origin (Shriver and Kittles 2004). Moreover, the mtDNA information should be complemented by the other mitochondrial single-nucleotide polymorphism (SNP) information, since they are haplogroup specific, usually outside the HVI and HVII regions. Besides technical and theoretical caveats, such an analysis should be carefully interpreted, since mtDNA results cannot be extrapolated to the rest of the genome, namely the paternal lineages or autosomal ancestry, inferred through the study of the Y-chromosome or ancestry-informative markers, respectively (Romanini et al. 2015). An extra difficulty with regard to the analysis of ancient genomes is related to the possible contamination of exogenous DNA, the presence of inhibitors of the PCR reaction, as well as the frequent absence of results that may be due to the poor state of the conservation of DNA due to fragmentation, oxidation, and hydrolysis, among others.

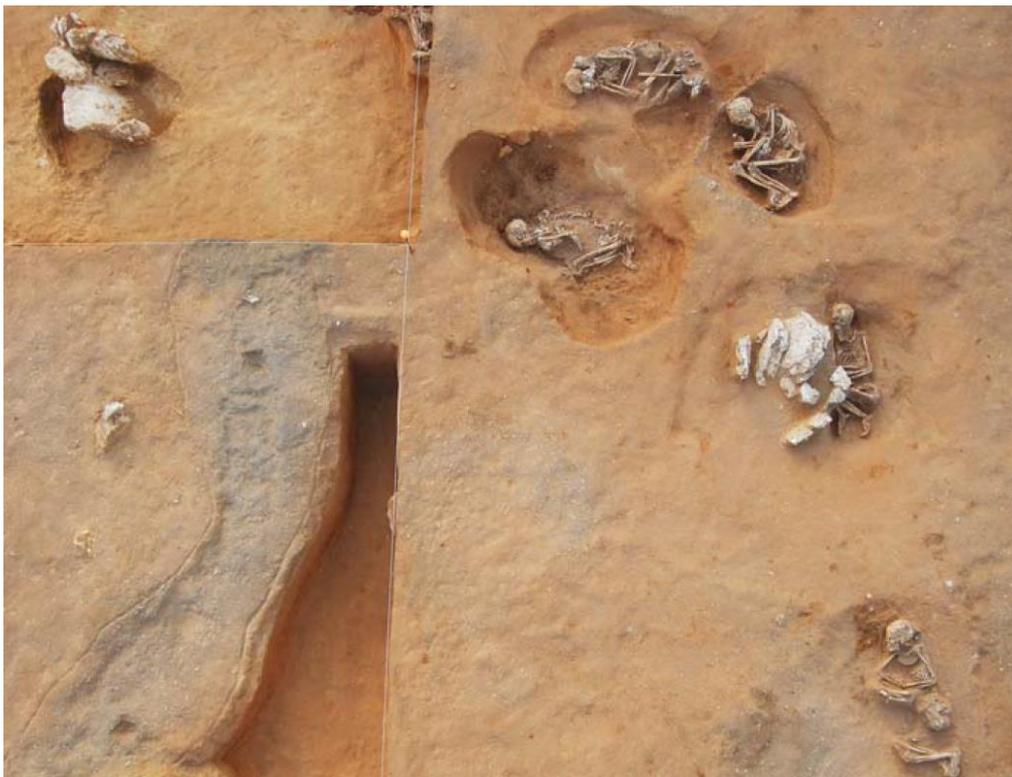
The purpose of the present work was to study 16 individuals considered archaeologically "Phoenicians" found in different burial places in the ancient city of Gadir (Cádiz, Spain), at a molecular level, in order to disclose their maternal biogeographical ancestry. To perform this, the molecular analysis of HVI and HVII mitochondrial regions, and other specific haplogroup polymorphisms were performed. Furthermore, the determination of a

possible kinship between the two individuals buried together was also an objective of this investigation.

#### *Archaeological Data*

In the present work six different parts of the ancient city of Gadir were studied (Supplementary Material Table S1 (S.M. Table S1)). This work will describe the most significant ones from an archaeological point of view, such as the necropolis of “Campo de Hockey” (“Hockey Field”), the Roman section and the “Solar do Antigo Teatro Cómico” (“Manor of the Old Comic Theater”).

One of these sections studied here, called “Campo de Hockey” (Cádiz, Spain), has revealed to have had a high population, and been verified in the lower area of the site as a singular and extensive Neolithic necropolis with 83 individuals. The typology of funerary structures is varied and ranges from the simplest burials (deposited directly in the ground or simple graves) (Figure 1), to the most monumental burial mounds or tombs. The burial ritual consisted of individual burials, with the subjects deposited in a fetal position, lying on their right or left side and with their hands placed at chest level or under their faces. Likewise, three double and two triple burials have been located. The necropolis is the result of careful planning. Despite the numerous individuals buried, the structures are not usually built on previous burials. The burial mounds and the vertical slabs (as a stele) of many of these burials would serve as signaling elements (Vijande et al. 2007).



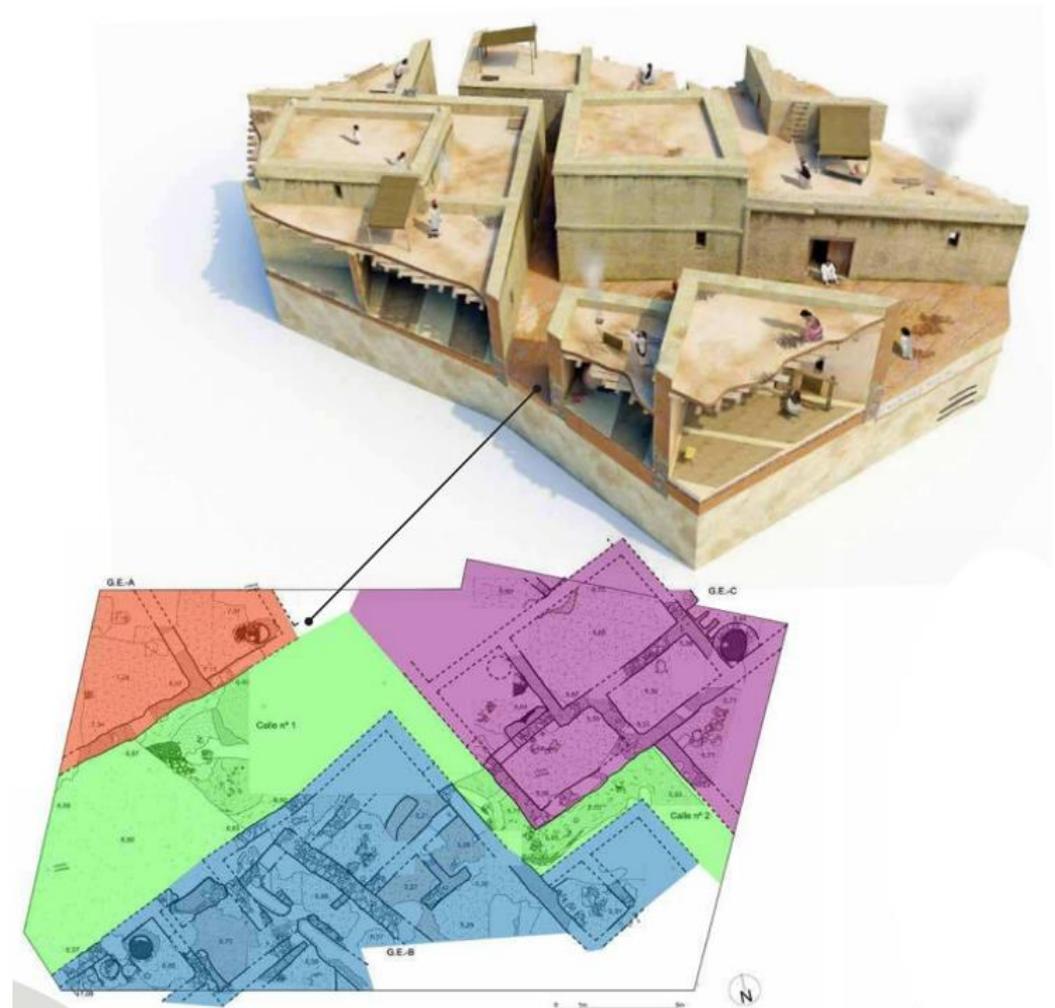
**Figure 1.** “Campo de Hockey” aerial view of various burials in a simple grave. Source: [Vijande et al. \(2007\)](#).

Despite the simplicity of the grave goods, the presence of some extremely interesting exotic objects, such as variscite, turquoise or amber, must be pointed out (Vijande et al. 2007).

Although the stratigraphy has delimited 10 periods of occupation of Gadir, the most interesting from an archaeological point of view belongs to the Roman and Phoenician periods. During the Roman periods, between the second half of the 2nd century B.C., important remains began to be found. In imperial times, from the 1st century, the remains

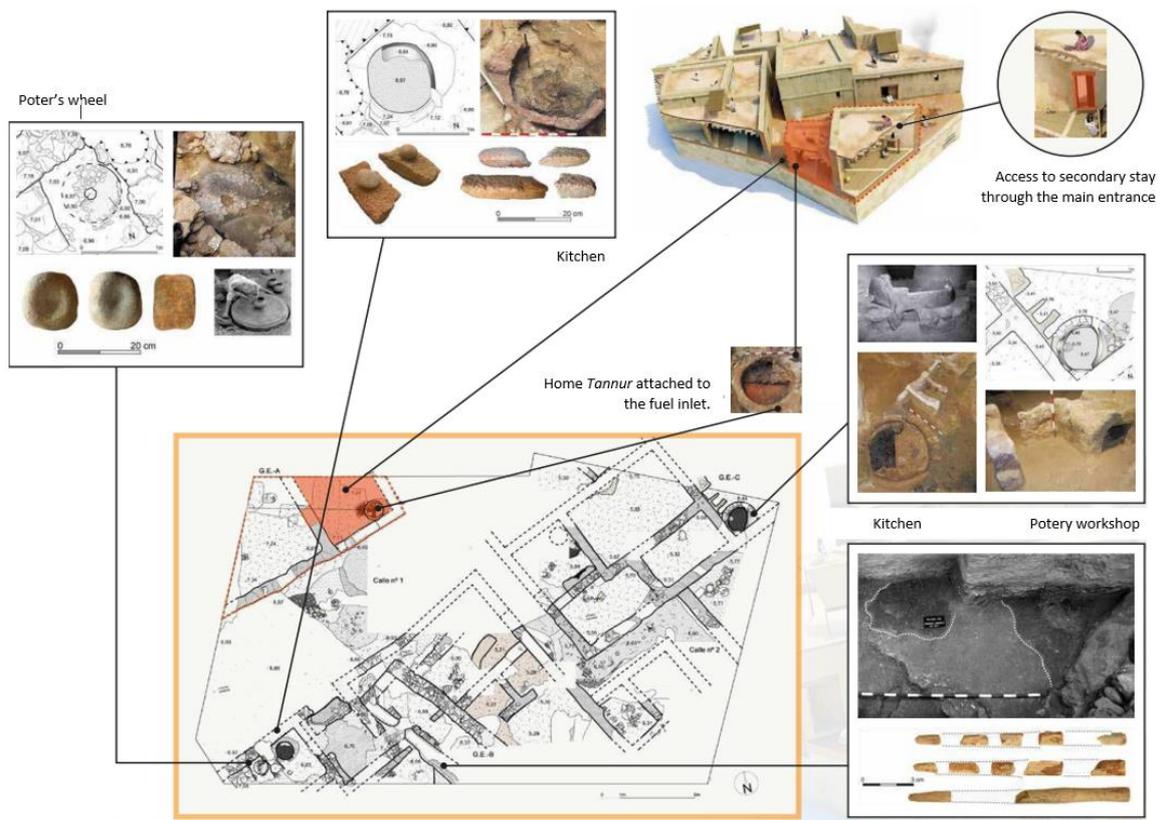
found give an idea of the complete occupation of the site. There are two buildings, which appear to have been part of a dry cleaner and a salting factory (Gener and Núñez 2015).

The other part of the site belongs to the oldest stage, the Phoenician Gadir, which, although there seem to be indications of a possible previous occupation, is from the year 820 B.C. to 720 B.C., when a large part of what was the Phoenician city was discovered. At the depth of 9 m, there are architectural remains of eight houses, distributed in two terraces and organized around two paved streets (Figure 2) (Gener and Núñez 2015).

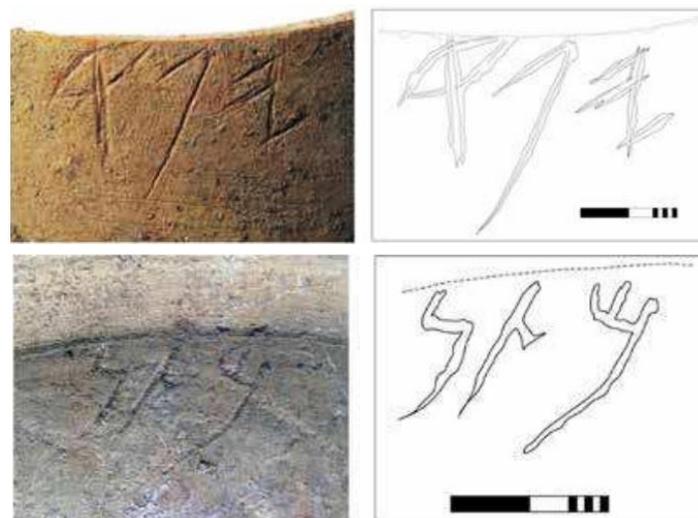


**Figure 2.** Planimetry of the Phoenician houses and 3D reconstruction, 820–760 B.C. Source: Gener and Núñez (2015).

In several buildings, the location of what would be kitchens with the remains of a *tannur*-type oven can be appreciated. Parts of the layout of the streets can also be seen, the first being the oldest and widest, and the second narrow and zigzag. The houses used to have a rectangular shape and could reach more than one floor. The main materials used for their construction were oyster stone, clay and lime. The distribution of each house is typical of Phoenician houses, with the main quadrangular room being larger and usually taller. The rest of the rooms were distributed around this one, such as the kitchen, workshops, or the rooms. In one of the houses, it was possible to observe a probable pottery workshop (Figure 3), where the remains of a potter's wheel, large containers filled with dye and bone, and ivory utensils for decorating ceramics were found. Another important finding was the discovery of archaeological elements in Phoenician writing (Figure 4).



**Figure 3.** Planimetry and various archaeological finds. Source: [Gener and Núñez \(2015\)](#).



**Figure 4.** Graphites with a Phoenician script. Source: [Gener and Núñez \(2015\)](#).

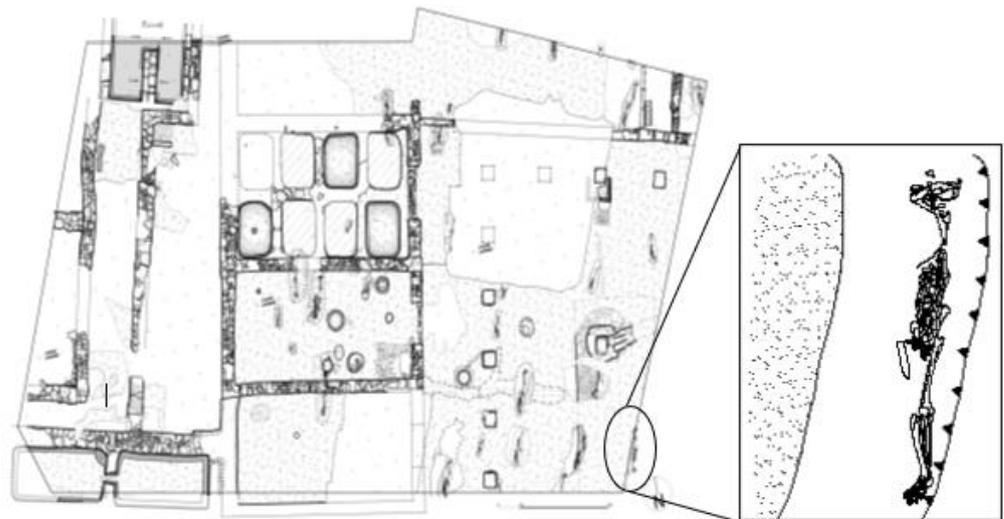
Below the Roman dry cleaner and salting factory, about 6–7 m into the north–west sector of the excavation, there was a Phoenician wall from the 6th century B.C. At a distance of 6–8 m from the wall, several individuals dating from Phoenician times appear. Among them, individuals 9, 10, 11 and 12 were studied in the present investigation.

## 2. Materials and Methods

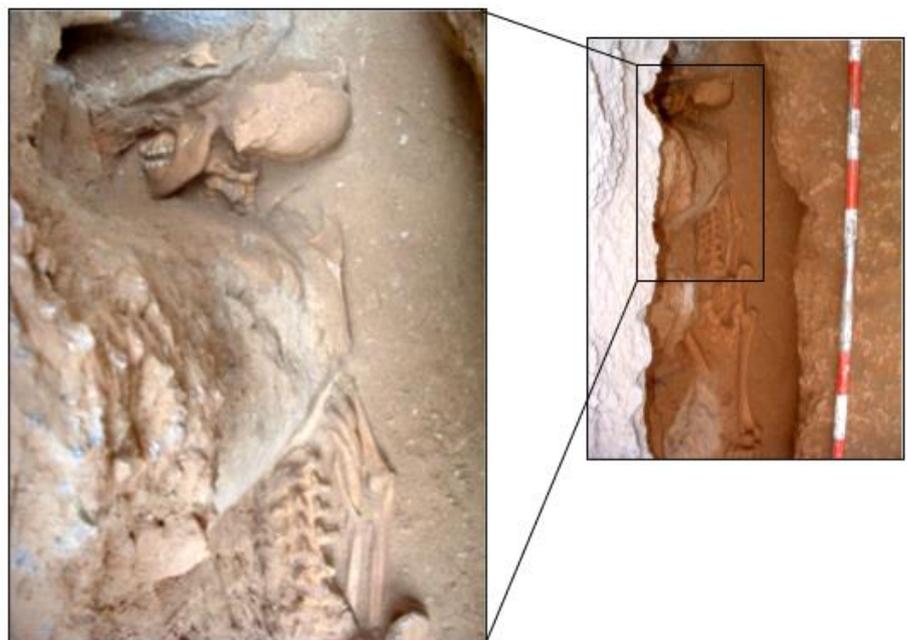
### 2.1. Anthropological Records

In this study, 16 individuals belonging to different periods were studied (S.M. Table S1), from the 5th–4th millennium B.C. to the 4th–5th A.D., all found in the region known during

Phoenician times as Gadir, modern Cádiz (Spain). The external condition of the human remains was not homogeneous, differing from individual to individual. Figure 5 shows individual 9 as an example, found in one of the sections of this study, the “Solar del Antiguo Teatro Comico”, together with the planimetry where he was found. In S.M., Figures S1–S3 the other three individuals found in this section, individuals 10, 11 and 12, can be seen. Additionally, in the Supplementary Material, there is a description of all of the individuals’ samples considered morphologically suitable for the present study (S.M. Table S1A,B). In general, the samples show considerable external deterioration, with some of them, mainly bone, being considerably porous. Regarding the dental samples, despite showing a certain degree of deterioration, the majority showed an intact color and appearance without fissures.



Plan of the salting factory from Roman times, in the NW sector of the excavation there was the Phoenician wall.



**Figure 5.** Planimetry and photographs of individual 9 found in one of the corners of the building. Source: JM Pajuelo and JM Gener Basallote.

## A Complex Burial: The Case of Individuals 8i and 8ii

Individuals 8i and 8ii were found buried together, and it was not possible to collect more information. For example, it was not possible to determine the individuals' sex by anthropological methods (S.M. Table S1).

### 2.2. Ancient DNA Analysis

#### 2.2.1. Sampling Process

In the present study 41 tooth samples were analyzed, corresponding to a total of 16 individuals (S.M. Table S1).

Due to the high chance of external contamination, all of the procedures were carried out in dedicated decontaminated rooms, where, prior to each analysis, the environments were disinfected with pure bleach, 70% ethanol, and ultraviolet radiation for at least 12 h. Genetic analyses were performed according to the authenticity criteria described by [Pääbo et al. \(2004\)](#). Depending on the availability of samples, two or three teeth from each individual were then selected to replicate the experimental process. Samples were selected to avoid pieces with cracks, cavities and caries, preventing the destruction of human genetic material by bacteria or fungi and contamination.

#### 2.2.2. DNA Extraction

A previous step common to both methods is the external cleaning of the bone and dental samples. This is carried out using aluminum oxide under pressure, using a sandblaster (Dentalfarm<sup>®</sup>). Subsequently, each sample was irradiated with UV ( $\lambda = 256$  nm) for 30 min on each side.

DNA extraction was performed with two distinct procedures, to compare the attained results: one sample with a destructive method (method A) and the other tooth with a non-destructive one (method B). The extraction methodology with the two methods is used routinely in the laboratory as a way of guaranteeing that different methods always produce the same results, and both methods have been previously proven to allow obtaining the same efficiency in the extraction of genetic material ([Gomes et al. 2015](#)). When access was obtained to a third tooth, the first two samples were analyzed with method A, and the third with B.

#### Method A: Destructive Procedure

After their external cleaning, samples were pulverized with a freezer mill and filled with liquid nitrogen. Then, the extraction technique was carried out following the guidelines published by [Rohland and Hofreiter \(2007\)](#). This procedure is based on the use of silicon dioxide and the use of three distinct buffers: extraction, which allows for the release of DNA from the cells; washing, which aims to separate the DNA from the other cell components; and finally a binding buffer that facilitated the association between silica and DNA.

#### Method B: Non-Destructive Technique

In this technique, the complete sample was processed without the pulverizing step. The extraction protocol was followed according to [Gomes et al. \(2015\)](#), also with the extraction, washing and binding buffers, used to perform the DNA extraction in method A.

#### 2.2.2.3. mtDNA Amplification

Two short overlapping sequences (175 bp and 170 bp) from mtDNA hypervariable region 1 (HVR1), as well as two overlapping sequences from HVR2 (119 bp and 100 bp) were analyzed, performing the amplifications with the QIAGEN Multiplex PCR Kit (Qiagen<sup>®</sup>). The amplification conditions used are those contained in the laboratory's standard work protocols for samples in an advanced state of degradation. Specifically: 95 °C 15 min; 94 °C 30 s; 55 °C 1 min 30 s; 72 °C 1 min (40 cycles); and 72 °C 10 min. Primer sequences used for the HVR1 and HVR2 amplification are described in Table S2. Additionally, a biological

sample from all the geneticists in the laboratory was also extracted and amplified to control any possible local contamination.

After amplification, all samples were visualized on a 1% agarose gel to see whether or not there was amplification, using an allelic ladder (Biotools<sup>®</sup>) and verify the fragment specificity. The amplified samples were then purified with ExoSAP-IT<sup>™</sup> PCR Product Cleanup Reagent (Thermo Fisher Scientific) and sent to MacroGen<sup>®</sup> for sequencing with the respective primers of each amplification, both forward and reverse. Finally, the sequence analysis was performed using the MutationSurveyor v4.0.9 software (SofGenetics<sup>®</sup>), noting not only the polymorphisms found, but also the quality of each sequence indicated by the software, from 0 to 100. From all the sequences analyzed for each individual, a consensus haplotype was determined (Table 1), where each polymorphism found was confirmed at least three times in independent amplification sequences.

**Table 1.** mtDNA haplotypes achieved for the 16 studied individuals, including the most probable maternal haplogroup.

Individual	mtDNA Position Range	mtDNA Haplotype	HAPLOGREP	EMPOP Frequency (17 March 2023)	Haplogroup-Specific SNP Result	Assigned Haplogroup	Consensus Haplogroup
1G	—	No result	—	—	—	—	—
2G	—	No result	—	—	—	—	—
3G	—	Without consensus	—	—	—	—	—
4G	—	Without consensus	—	—	—	—	—
5G	—	Without consensus	—	—	—	—	—
6G	—	Without consensus	—	—	—	—	—
7G	123–320; 16,105–16,399	195C 198T 263G 309.1C 16269G 16298C	HV0b 86.18%	HV0b 1/42,210	14766C 7028T	HV0 HV0	HV0
8iG	8–128; 16,105–16,280	72C	HV0b 100%	HV0b 413/40,258	14766C 7028T	HV0 HV0	HV0
8iiG	10–390; 16,105–16,399	72C 195C 198T 263G 309.1C 16269G 16298C	HV0b 89.02%	HV0b 0/39,355	14766C 7028T	HV0 HV0	HV0
9G	8–73; 127–391; 16,105–16,399	72C 195C 198T 263G 315.1C 16269G 16298C	HV0b 89.02%	HV0b 1/39,355	7028T	HV0	HV0
10G	55–127; 135–378; 740–769; 16,231–16,399	73G 263G 315.1C 750G 16278T 16362C	L3b 100%	L3b 17/4300	10873C 10398A 8701A	L3 No result No result	L3
11G	8–390; 16,105–16,399	152C 263G 315.1C	H1c	H 290/39,355	7028C	H	H
12G	126–390; 16,105–16,399	195C 198T 263G 309.1C 16269G 16278T 16298C 16362C	HV0b 80.1%	HV0b 0/41,708	7028T	HV0	HV0
13G	—	Without consensus	—	—	—	—	—
14G	—	No result	—	—	—	—	—
15G	8–390; 16,105–16,399	263G 315.1C	H2a2a 100%	H 1237/39,355	7028C	H	H
Researcher C.	8–390; 16,105–16,399	T152C, A263G, 309.1C, 315.1C, T16304C	H5	H5	—	—	—
Researcher S.	8–390; 16,105–16,399	A55G, C150T, T239C, A263G, 309.1C, 309.2C, 315.1C, T16362C	H6a	H6a	—	—	—

#### 2.2.2.4. mtDNA Haplogroup Estimation

The estimation of each haplogroup was performed as follows:

(a) Consulting the phylogenetic tree PhyloTree, using HaploGrep (Kloss-Brandstatter et al. 2011; van Oven 2015). This online application indicates an estimated probability of the individual belonging to a specific haplogroup, according to his/her DNA sequence.

(b) Assignment of each haplotype to a biogeographical population (Prieto et al. 2011) using the EDNAP Mitochondrial DNA Population Database (EmPOP) (Parson and Dur

2007), which was also accessed for estimating the most probable biogeographical ancestry of the sample.

(c) Accessing the phylogenetic tree PhyloTree directly (van Oven 2015; van Oven and Kayser 2009) and checking the defining mutation(s) of each haplogroup.

### 2.2.3. Specific Haplogroup SNP Amplification

A set of haplogroup-specific SNPs located in other mitochondrial regions was selected to confirm the detected haplogroups, only considering the individuals with HV1- and/or HV2-positive results. The amplification was performed according to Gamba et al. (2012). Table S3 displays the studied SNP results.

The results were considered consistent only when it was possible to obtain the same sequence by at least two independent amplifications from the same tooth extract. Such a procedure was carried out in all samples, and full concordance of the results was checked.

### 2.3. Statistical Analysis

A multinomial statistical analysis was carried out by the Calculation Center of the Teaching and Research Support Center of University Complutense of Madrid (Spain), considering the obtained set of results (5 individuals belonging to one mitochondrial haplogroup, 2 individuals to another, and 1 individual to a third group), and the probability that such a group had been sampled from a particular population. Therefore, two different analyses were computed. The first one was performed with three modern metapopulations (African, European, and Eastern), and the second one with specific populations within the former three: “French”, “Sardinian”, “North Italian”, “Tuscan”, “French Basque”, “Bedouin”, “Mandenka”, “Yoruba”, “Mozabite”, “Druze”, “Palestinian”, and “Yakut” (Emery et al. 2015). To compute the relative population frequencies, haplogroup information was consulted by Emery et al. (2015) in both cases.

To evaluate the obtained results for the metapopulations, a likelihood ratio (LR) approach was undertaken. The LR approach consists of the calculation of a quotient between probabilities of the same event, considering two exclusive hypotheses (H1 and H2, for example, H1: This set of eight individuals has a European biogeographical ancestry, and H2: This set of eight individuals has an African biogeographical ancestry). Thus, LR was calculated as:

$$LR = \frac{(Evidence | H1)}{(Evidence | H2)}$$

## 3. Results

### 3.1. Result Quality

Although the archaeological reports stated that, in general, the samples were in an acceptable condition, during the cleaning and extraction procedures, some fragility and poor integrity were observed. The quality of the sequences analyzed, obtained through the MutationSurveyour v4.0.9 software (SofGenetics®), varied for method A between 0 and 54, and for method B between 0 and 63, so we consider the performance of both methods to be similar. Thus, there are more accentuated differences between individuals than between methods. Normally, sequences with a quality between 0 and 20 had to be repeated more times for a correct reading of the polymorphisms, in some cases having to repeat four times. For example, for individual 11 G, it was necessary to perform four DNA amplifications to obtain legible and satisfactory quality sequences.

### 3.2. Biogeographical Ancestry

In Table 1, the mtDNA haplotypes achieved for the 16 studied individuals are shown. Such information refers to A and B extraction techniques and shows three possible results: “no result”, when it was never possible to obtain a sequence; “without consensus”, when positive, but different results were obtained in distinct amplifications considering the same or different samples of the same individual. When it was possible to define a haplotype,

the consensus sequence was described. Table 1 also shows the mtDNA profiles of the researchers who worked with the samples in the laboratory. No matches were observed when comparing researchers with archaeological samples.

#### Haplogroup-Specific SNP Results, Haplogroup Assignment, and Phylogenetic Analysis

Considering the 16 individuals, eight produced positive results. However, in several cases where it was not possible to assign just one haplogroup using HaploGrep (Kloss-Brandstatter et al. 2011; van Oven 2015), the EmPOP (Parson and Dur 2007) database was accessed to point out the most probable biogeographical ancestry.

Considering the mitochondrial transmission pattern (excluding cases of heteroplasmy), one individual will exhibit only one haplotype, belonging to a specific biogeographical ancestry. In those cases, where both HaploGrep and EmPOP indicate more than one haplogroup possibility, the obtained sequences in fact exhibit mutations that are present in more than one maternal lineage.

The results of the haplogroup-specific SNP analysis can also be consulted in Table 1, specifying the SNP result, as well as the haplogroup indicated by the SNP result.

#### 3.3. Statistical Analysis

Concerning the statistical analysis, S.M. Table S4 (Supplementary Material) contains the results from both multinomial studies. Here it is possible to observe that the most probable origin for the considered set of individuals was the Eastern metapopulation (probability =  $9.2 \times 10^{-7}$ ). However, when dealing with specific populations, the highest result was obtained for the Mozabites (probability =  $2.6 \times 10^{-5}$ ).

Taking into account the three metapopulations and the LR calculation among them, the highest LR value indicates that it is more probable to obtain this set of individuals from an Eastern metapopulation (LR =  $1.2543 \times 10^5$ ) than from an African or European population (Table 2).

**Table 2.** LR values obtained for the biogeographical ancestry determination.

H1	H2	LR
African	European	10,561.3604
Eastern	African	11.8762
Eastern	European	125,428.8424

## 4. Discussion

### 4.1. Results of the Quality and Authenticity

Apart from the recommended procedures, when dealing with ancient and/or critical DNA (Gomes et al. 2019), three methods were performed in the present work to verify signs of contamination in the final sample sequences. The first one was the confirmation of a total absence of DNA amplification in negative controls; the second procedure was the direct comparison between obtained results from samples and the research team haplotypes. However, mutations 263G or 315.1C are quite frequent in modern Europeans, though we have no information about their frequency in past populations. Therefore, the third process to detect signs of contamination was based on the observation of each polymorphism in the samples, in at least three independent amplifications.

On the other hand, the deterioration of the samples was also verified in the number of amplifications necessary to obtain each fragment and to confirm each polymorphism. Indeed, fewer amplifications were necessary in the case of the 119 bp fragment than in the case of the 255 bp fragment (S.M. Table S2).

Concerning the comparison between both extraction methods, on the one hand, the fact that the same sequences were obtained with particularly different extraction methods increases the confidence in the obtained results. On the other hand, the fact there were more differences between individuals than between methods is also understandable, since,

as mentioned before, the state of conservation of many of the biological samples was quite precarious, especially the oldest ones. This is also understandable, taking into account that the individuals would be in similar physicochemical conditions, so one of the factors that could affect the conservation of the genetic material would be the passage of time and the consequent loss of genetic information (Gomes et al. 2019). Excepting individuals 13G and 14G, samples with positive results were those belonging to the periods between I B.C and IV–V A.D, the most recent samples. Those from previous periods did not show positive results, or it was not possible to obtain a consensus result.

#### 4.2. Biogeographical Ancestry

##### 4.2.1. The HV0 Lineage

Five of the eight individuals exhibit the HV0 mitochondrial lineage. The HV mitochondrial haplogroup is a major clade within haplogroup R0, characterized by the 14766C mutation, which comprises at least 18 subclades (De Fanti et al. 2015), including HV0. Analyzing the mitochondrial information provided by Emery et al. (2015), it is important to note the frequency of the HV mitochondrial haplogroup in the Near East, considering the current localization of ancient Phoenicia, i.e., Lebanon. Although genetic drift episodes can indeed change genetic frequencies, the reconstruction of an eventual genetic portrait of the ancient Phoenicians could be approached through the analysis of modern population(s). Bottleneck episodes, such as catastrophic events of natural origin (earthquakes, seaquakes, for example) or from human nature (such as war events) lead to the alteration of genetic frequencies in a given place, due to population movement, as well as the deaths that it causes. For this reason, it is particularly interesting that one of the lineages found in the studied individuals from Phoenician times, HV0, coincides with one of the lineages of which the frequency is currently the highest in Lebanon, ancient Phoenicia. Even knowing that the probability of the occurrence of bottleneck-type events such as those mentioned above is very high, there seems to be a coincidence between the individuals of the Phoenician period of Gadir and one of the most prevalent lineages in Phoenicia (modern Lebanon).

Analyzing the Iberian Peninsula and according to Barral-Arca et al. (2016), the actual frequency of the macrohaplogroup R is higher in the northern half of the Iberian Peninsula than in the South. In line with previous information, the frequency of the HV0 haplogroup finds a peak frequency in the actual Basque region (North of Spain) (Barral-Arca et al. (2016)), but it is not a very frequent lineage when considering the whole Iberian Peninsula (Nogueiro et al. 2015). Although Mairal et al. (2013) found a moderate HV0 lineage frequency in Miranda do Douro, Portugal (8.3%), and Zamora, Spain (8.4%), Nogueiro et al. (2015), when investigating Jewish Sephardic lineages in Portugal, a significant incidence of this mitochondrial lineage was found. Indeed, it is mentioned that 93% of the analyzed mtDNA genomes in the Jewish community of Belmonte (Portugal) correspond to the HV0b lineage (Nogueiro et al. 2015). Historically, the migratory route followed by Sephardic Jews, extended along the coast of the Mediterranean and could have accompanied the Phoenician and other maritime dispersions, in accordance with the proposal for the dissemination of the Y-chromosome J haplogroup, in particular, subgroup J2 advanced by Di Giacomo et al. (2004) and Zalloua et al. (2008). This is relevant data, since both Phoenicians and Jews have a similar origin (Padró 2012). Israelites seem to have been associated with the Canaanite culture. This relationship manifests itself in several aspects: technological, linguistic, and ethnological. The Canaanites, indigenous people of that area, were Semites and resulted possibly from a mixture of different groups: the Amorites, nomadic shepherds, also Semites; the Hittites, non-Semitic, who occupied part of Syria and Mesopotamia; the Amalekites, nomads from southern Palestine; and the Philistines, who occupied the Mediterranean coast south of Jerusalem (Aubert 2001; Levy-Coffman 2005). In the Iberian Peninsula, the most ancient discovery concerning Jewish evidence has been related by Graen (Friedrich-Schiller-Universitaet Jena 2012), and concerns “an excavation site in the south of Portugal, close to the city of Silves (Algarve)”, dated 482 A.D. This probably

means that both Phoenicians and Jews coincided in the Iberian Peninsula, beyond their geographical proximity in the Near East.

However, considering the dating of the samples, four out of the five individuals (8iG, 8iiG, 9G and 12G) belong to the Roman Empire period in the Iberian Peninsula, and according to [Di Bernardo et al. \(2009\)](#), the HV0 haplogroup could also be found in the ancient Roman Empire. This could signify that these human remains were Romans who lived in Iberia and who adopted a Phoenician lifestyle.

Finally, considering not only the genetic information, but also evidence found in the burial sites, such as the type of tomb or the ornamental ceramics, all data seem to indicate that the buried individuals have a Phoenician ancestry.

#### **Multiple Burials: The Case of 8iG and 8iiG Individuals**

One of the objectives of this work was to detect a (possible) biological link between 8iG and 8iiG. Since they were found buried together, the genetic study aimed to help understand if they were buried together due to a biological kinship between them. By comparing the obtained sequences from both individuals, it was possible to conclude that they do not share the same maternal lineage. It is possible to discard biological relations, such as mother–son/daughter; siblings; maternal half-siblings, or other maternal kinship. Therefore, there are three main hypotheses: (a) the individuals are related by a paternal kinship, such as father–son/daughter, or paternal grandparent–grandson/granddaughter; (b) the individuals were united by marriage; (c) there were social, but not biological bonds between both individuals. From the molecular point of view, it is impossible to state what kind of relationship they shared.

#### **4.2.2. The H Lineage**

Considering individuals 11G and 15G, they are both dated as belonging to the IV–V A.D centuries, and it was possible to determine that the most probable macro-haplogroup for them both was H. This haplogroup, characterized by G2706A and T7028C polymorphisms, has an important representation in western Eurasia ([Roostalu et al. 2007](#)) nowadays. [Roostalu et al. \(2007\)](#) indicate that in the present Lebanese population, the haplogroup H1 has a considerable frequency ([Roostalu et al. 2007](#); [Emery et al. 2015](#)). Such evidence could support a Phoenician lineage in both 11G and 15G individuals.

Nevertheless, the H haplogroup also has a significant frequency in Europe ([Emery et al. 2015](#); [Zalloua et al. 2018](#)), and regarding the actual Iberian Peninsula, the H haplogroup has a higher frequency in the Atlantic facade, decreasing its presence towards the Mediterranean and Andalusian regions ([Barral-Arca et al. 2016](#)). The evidence of this haplogroup is also described, not only in present European individuals but also in past populations, such as the ancient Roman population ([Töpf Ana et al. 2006, 2007](#); [Gamba et al. 2008](#); [Di Bernardo et al. 2009](#); [Martiniano et al. 2016](#)). It should also be considered that before the Roman Empire, the natives of the Iberian Peninsula had close contact with the earlier Phoenician settlers ([Tsirkin 1997](#); [Zalloua et al. 2018](#)).

Another interesting fact has to do with the anthropological record that indicated that 11G had a probable African origin. [Ottoni et al.](#) mention that H1 is one of the most prevalent haplogroups in North Africa ([Ottoni et al. 2010](#)). They also suggest that this haplogroup could have been carried out by migration from Europe, especially from the Iberian Peninsula to North Africa.

In the study performed by [Zalloua et al. \(2018\)](#) of possible Phoenician individuals from the island of Ibiza (Spain), this macrohaplogroup predominates, which may indicate, on the one hand, that there was a deep presence of European “natives” in Phoenician cities, or that, in fact, it is a lineage that was also common in the Phoenician population.

Thus, considering all the information for 11G and 15G individuals, it is not possible to simply discard any hypothesis. On the one hand, we have archaeological evidence that points to a Phoenician culture, yet, on the other hand, the molecular evidence does not allow for the Near East ancestry to be discarded, nor Tartessic, Roman or other European maternal origins. Regarding 11G, the African hypothesis should also be taken into account.

#### 4.2.3. The L3 Lineage

The L3 lineage (characterized by the A769G, A1018G and C16311T mutations) is one of the African macro-haplogroups, which is currently common in Northeast Africa (Harich et al. 2010), in contrast with the other two African macro-haplogroups (L1 and L2) (Kujanova et al. 2009). According to Wallace et al. (1999), L3 forms the bridge between African and European–Asian mtDNAs, being more related to the Eurasian haplogroups than to the African clusters, L1 and L2 (Maca-Meyer et al. 2001). In fact, two of its sub-haplogroups—M and N—diverged and are carried by most humans outside of Africa (Wallace et al. 1999).

Considering our results, the individual 10G shows L3b lineage. The presence of this haplogroup in one individual found in the South of the Iberian Peninsula could be explained by two hypotheses. First, the trade of the goods between North Africa and Phoenician cities, made the migration of people between both places plausible; second, the slave trade among Phoenician colonies (Fernández Uriel et al. 2000).

#### 4.3. Statistical Analysis

The statistical analysis was based on the hypothesis that this set of individuals has been sampled together, as if they were all contemporary or, in other words, that the haplotype of the most recent individual is the same as that of his ancestor, who was a contemporary of the most ancient individual. This can be assumed since mitochondrial DNA is a lineage marker, and it is not expected that the haplotype would change along the same lineage over time. Even if mutational events occur, the expected result would be the appearance of private mutations and should not affect the individual's haplogroup classification.

In general, the results support an Eastern origin for this set of individuals. Nonetheless, when dealing with specific populations, the most probable ancestry is a North African population. Considering table S.M. Table S4, it is possible to observe that only three specific populations have the H, HV0 and L3 haplogroup frequencies greater than 0.0001: Bedouin, Mozabite, and Palestinian populations. The Mozabite population, a Berber ethnic group inhabiting the northern Sahara, was the one presenting higher frequencies among the three metapopulations, for the three considered haplogroups. Hence, assuming the plausibility of the presented hypothesis and considering the Mozabite geographical location, this population could also be a possible origin for these individuals.

Finally, our results contrast with those obtained by Matisoo-Smith et al. (2016), who identified the U6 haplogroup, pointing to European ancestry, while in our study we identified three main lineages (HV0, H and L3), which could be ascribed to Europe, Near East and North Africa.

The analysis of these individuals does not allow the peremptory confirmation that their origin was from the Near East. The data that best support this theory is the archaeological evidence found in burial places. However, even though these individuals are not contemporaneous, we can verify that, for example, the maternal lineage HV0 and H are transversal throughout the different periods studied in this work and are still present in the modern Lebanese population (Emery et al. 2015), where ancient Phoenicia was situated.

Although the genetic evidence is not homogeneous for all individuals, the archaeological evidence seems to demonstrate that individuals, even those not belonging to a Phoenician biological “lineage”, maintained the same rituals and traditions, even in terms of infrastructure and ways of writing. Gomes et al. (2021) discuss what the term “Family” means, explaining not only the biological, but also its social meaning. In this case, the same criterion could be applied, considering the fact that individuals identifying themselves as Phoenicians could not be related to a genetic lineage, but to oral and written tradition and to the feeling of belonging to a certain society.

## 5. Conclusions

Of the analyzed individuals, eight of them generated positive results. Five were classified within the HV0 mitochondrial lineage, the frequency of which is fairly considerable

in the Near East. On the other hand, two of the eight belong to the macro-haplogroup H, which has a valuable representation in western Eurasia nowadays. Finally, the last analyzed individual presents the L3b lineage, one of the African macro-haplogroups currently widespread in Northeast Africa. In the present study, it is extremely complex to be able to associate a particular lineage identity with the individuals in question. Given the period to which they potentially belong, they may be descendants of the Phoenician ancestral population, or they may already, at a genetic level, come from another gene pool, from the Iberian Peninsula or not, considering the different Roman trajectories along its expansion. In general, the results support an Eastern origin for this set of individuals, reinforcing the theory of a possible Phoenician origin. However, due to their historical period, in some cases, it was not possible to discard a Roman origin.

The results obtained in this research are notably significant. In some cases, the archaeological record can demonstrate that, despite belonging to a distinct biological pool, individuals maintain their ancestral rituals and traditions, feeling that they belong to the ancient Phoenician genealogy.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/genealogy7020027/s1>, S.M. Table S1—Morphological description of the individuals that were subjected to genetic analysis; S.M. Table S2. Primers used for the HV1 and HV2 mtDNA region amplifications; S.M. Table S3. Haplogroup-specific SNPs set with their respective sequence; S.M. Table S4. Multinomial-obtained results for the three considered metapopulations (African, Europe and Eastern populations) and the thirteen specific populations. The presented frequencies were obtained by consulting [Emery et al. \(2015\)](#). S.M. Figure S1. Planimetry and photographs of individual 10 found in the “Solar del Antiguo Teatro Cómico”. Source: JM Pajuelo and JMGener Basallote. S.M. Figure S2. Planimetry and photographs of individual 11 found in the “Solar del Antiguo Teatro Cómico”. Source: JM Pajuelo and JMGener Basallote. S.M. Figure S3. Planimetry and photographs of individual 12 found in the “Solar del Antiguo Teatro Cómico”. Source: JM Pajuelo and JMGener Basallote.

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