Insights into Punic genetic signatures in the southern necropolis of Tharros (Sardinia)

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ABSTRACT

Background: Phoenician and Punic expansions have been protagonists of intense trade networks and settlements in the Mediterranean Sea.

Aims: The maternal genetic variability of ancient Punic samples from the Sardinian necropolis of Tharros was analysed, with the aim to explore genetic interactions and signatures of past population events. **Subjects and methods:** The mtDNA HVS-I and coding region SNPs were analysed in 14 Punic samples and 74 modern individuals from Cabras and Belvì (for which the HVS-II region was also analysed). The results were compared with 5,590 modern Euro-Mediterranean sequences and 127 ancient samples. **Results:** While contemporary groups fall within the genetic variability of other modern Sardinians, our Punic samples reveal proximity to present-day North-African and Iberian populations. Furthermore, Cabras and Belvì cluster mainly with pre-Phoenician groups, while samples from Tharros project with other Punic Sardinian individuals.

Conclusion: This study provides the first preliminary insights into the population dynamics of the Punic site of Tharros. While the number of currently available samples does not allow definitive inves-tigation of the connection with indigenous Sardinian groups, our results seem to confirm internal migratory phenomena in the central-western Mediterranean and female participation in the Punic mobility.

KEYWORDS

Ancient DNA; mtDNA; archaeology; Tharros; Punic Sardinia

Introduction

The history of Sardinia has been for decades the subject of interest for a large number of population-genetics studies. In fact, the unique genetic heritage of its population and its relative isolation have made present-day Sardinians an extraordinary reservoir of specific genetic signatures (Cavalli-Sforza et al. 1994; Calò et al. 2008; Contu et al. 2008; Ghirotto et al. 2010; Francalacci et al. 2013; Di Gaetano et al. 2014; Sidore et al. 2015; Modi et al. 2017; Olivieri et al. 2017; Chiang et al. 2018). Furthermore, new sources of evidence stemming from the possibility of analysing DNA from ancient human remains have remarkably increased our knowledge of the past genetic ancestry and population pre-history (Lazaridis et al. 2014; Sikora et al. 2014; Allentoft et al. 2015; Haak et al. 2015; Mathieson et al. 2015; Olalde et al. 2015; Fu et al. 2016; Hofmanová et al. 2016; Antonio et al. 2019; Mathieson et al. 2018; Fernandes et al. 2020; Marcus et al. 2020). In this framework, Sardinia has often been modelled as being the best present-day representation of the Neolithic ancestry established during the early expansion of farmers into Europe, who then remained isolated from the subsequent population movements that occurred through the continent during the Bronze Age. Recently, a large survey of genome-wide ancient DNA data from Sardinian archaeological sites spanning from the Neolithic to the Medieval period, specifically highlighted the genetic affinity between the Middle Neolithic samples of Sardinia and the early Neolithic peoples of Europe and the Mediterranean, further suggesting a period of relative isolation with no signals of external admixture through to the Nuragic period (Marcus et al. 2020). Importantly, Sardinian individuals from the Nuragic period did not reveal any marked shift in ancestral composition, instead showing genetic continuity from the Middle-Neolithic into the Bronze Age, coupled with the absence of Yamnaya-Steppe-related ancestry ("Steppe"), which suggested genetic isolation with respect to Bronze Age mainland population movements. Evidence of novel ancestry components not observed in the earlier ancient Sardinian individuals instead appeared after the Nuragic

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period. Beginning with individuals from Phoenician and Punic sites, the analysed post-Nuragic ancient Sardinian samples indeed showed significant contributions from "Eastern-Mediterranean" and later "Northern Mediterranean" genetic components, evidencing an increased variation in ancestry possibly reflecting complex post-Nuragic gene-flows in the genetic history of the island (Marcus et al. 2020). In particular, local heterogeneity in the patterns of ancestry has been observed between post-Nuragic individuals with higher contributions from the earlier local ancestry and other samples showing the influx of novel components, paralleled by an increased diversity in haplogroup composition. A previous study that analysed mitogenomes from a large set of modern and ancient Sardinian samples (Olivieri et al. 2017) identified a set of Sardinian-Specific Haplogroups (SSH) suggesting some lineages to possibly be indicative of a pre-Neolithic origin and most of the other instead coalescing in Neolithic, Nuragic and post-Nuragic periods, thus arguing for a complex scenario in the dynamics of isolation and gene-flow into the island. Accordingly, a research study that specifically analysed complete mitogenomes in a Sardinian Phoenician settlement to explore the relationship patterns between local Sardinian and Phoenician or Punic groups, revealed both evidence of lineages in continuity from pre-Phoenician local populations, as well as new non-indigenous mitochondrial haplogroups, possibly indicating the movement of women and the integration with local groups (Matisoo-Smith et al. 2018).

In recent years, there has been an important increase in investigations into ancient DNA from the anthropological remains of Phoenician and Punic funerary settlements (Zalloua et al. 2008; Matisoo-Smith et al. 2016; Matisoo-Smith et al. 2018; Zalloua et al. 2018). Since these populations were the protagonists of many commercial and colonial expansion activities between the two basins of the Mediterranean Sea as of the First Iron Age, the ability to identify the origins of migration flow areas is of great interest. If we consider the most ancient phase of such movements, from a strictly archaeological point of view, it is quite challenging to distinguish the "Phoenicians" from the other populations of the Syrian-Palestinian coast, which include the Syrians, Aramaeans, or Philistines, each having enjoyed wide-ranging mobility. First of all, it is difficult to ethnically ascribe the handicraft productions that constitute the material evidence of these populations' existence, given the similarities in technological skills and productive and stylistic orientations. Secondly, it is unclear whether the ship crews defined as "Phoenician," who were responsible for the transport of various products from East to West, were in fact composed of Phoenician coastal city-state inhabitants as they are conventionally understood to be. Rather, the crews may well have been peoples of Syria, Palestine, Cyprus, or other Aegean contexts. It must also be considered that the composition of maritime crews changed between the different ports affected by Mediterranean traffic, just as it is likely that goods changed between one port of call and another. In the more advanced phases, following the Phoenician foundation of Carthage in North Africa and the main settlements in South Sardinia and the Iberian Peninsula, the Levantines were replaced by the main populations of the central-western Mediterranean. Starting from the second half of the 8th century BC and in the 7th century, this phenomenon took shape in the autonomous colonial expansion of Carthage, Sulci, and Cadiz (Secci 2019). These complex dynamics are also found in the case of Tharros (Fariselli 2017) at the end of the Sinis Peninsula in Sardinia (Figure 1). In fact, archaeological documentation attributes this urban context to the Carthage initiative in the 7th century BC (Fariselli 2018). At an earlier age, the area was affected by a network of exchanges between merchants from the East, the Aegean in particular, and indigenous communities. These Nuragic groups of the First Iron Age are recognisable in the coastal village of Su Murru Mannu and the imposing site of Mont'e Prama (Usai 2014). The contact between the Nuragic and Levantines in Sinis does not seem to have given rise to stable settlements, precisely because the oldest Tharros urban system is only found at a later stage, when, for reasons still undetermined, the indigenous population is no longer well-observed or distinguishable in archaeological data. However, the results of archaeogenetics are inevitably unclear if we try to confirm the Carthaginian origin of the first urbanised communities in the central Mediterranean, such as Tharros. With Carthage being a city founded in North Africa by the Phoenicians of Tyre, it is difficult to genetically distinguish a Phoenician of Tyre from a second-generation Phoenician of Carthage for the most ancient phases, especially considering the very wide diffusion of Mediterranean haplogroups, and the fact that the mixture between Phoenicians and indigenous North African populations is not historically attested to before the 6th–5th century BC. A further criticality regarding the Tharros population's genetic physiognomy since the 7th century BC involves clarifying the encounters with possible indigenous groups. In particular, the traces of a potential ethnic mixture with the Sardinians from the beginning of the colonial foundation are not easily distinguishable, because the first inhabitants of Tharros mainly used cremation as a funeral practice (Meli et al. 2020). Thus, since the success rates of DNA extraction from burned remains are relatively low, a possible admixture between the first Carthaginian settlers and the indigenous Sardinians would be very difficult to prove. It goes without mentioning that this mixture cannot currently be proven by archaeology, unlike other coastal and inland centres of Phoenician Sardinia. The preservation of DNA in such remains depends on several taphonomic factors, including *in primis* the burning temperatures (Gaudio et al. 2019) and the availability of particular skeletal elements, like petrous bone, that can contain higher endogenous ancient DNA yields than those obtained from other bones (Gamba et al. 2014; Pinhasi et al. 2015). Moreover, the two areas of the necropolis identified and excavated in Tharros, the smaller ones in the north and the larger and more monumental ones in the south of the Sinis peninsula, have been seriously compromised by past clandes-tine profanation. This is especially true for the funerary contexts of the inhumated, which usually held stores of precious jewel-lery. It is therefore difficult to orient the genetic analysis towards the foremost archaeological questions.

In this study, we have however the possibility to analyse samples dated from the Middle to the Late Punic period from the Southern necropolis of Tharros in Sardinia with the aim to study the first (available) genetic contribution to the past population dynamics in this important archaeological site, by further combining ancient DNA data with new results from modern population samples in order to understand the composition of maternal genetic variability through time.

Materials and methods

Archaeological site and ancient samples

A total of 14 ancient Punic samples (Supplementary Figure S1 and Supplementary Table S1) were collected from the southern necropolis of Tharros (Supplementary Figure S1), located on the Capo San Marco area in the Sinis Peninsula of Sardinia (Fariselli 2014; Fariselli 2017). The sampling was carried out during the archaeological campaign of excavation performed in 2014 under the direction of Prof. Fariselli of the University of Bologna (Fariselli 2014). The ancient samples were collected from the archaeological excavation under Ministerial Concession 2012-2016, DG. 3804 Class. 34.31.07/345.1, 5th of April 2012.

The selected samples are from rectangular pit tombs dug into the rock, dated between the Middle and Late Punic period. Although disjointed, the inhumated skeletal remains recovered from tomb A2 seem to be stratigraphically consistent with a set of intact ceramic vases dated to the 5th century BC (Fariselli 2017). The osteological material from tomb Z is associated with heterogeneous and non-diagnostic ceramics, allowing for only a generic context between the 5th and the 3rd century BC (Fariselli 2017). The collected human specimens particularly consisted of disarticulated and fragmented human bones, including long bones and loose teeth.

The sampling was carried out with stringent *in-situ* procedures to minimise the risk of contamination and increase DNA preservation (Pruvost et al. 2007; Bollongino et al. 2008; Fortea et al. 2008; Llamas et al. 2017). In particular: (i) all ancient specimens were collected using disposable lab coat, sterile gloves, face mask, hair net, over-shoes and all the equipment used was decontaminated with 5% NaClO and Ethanol before and after each sampling; (ii) the freshly excavated specimens were stored in specific plastic bags (annotated with sample description, location, number of burial and stratigraphic unit) and delivered to the laboratory of analysis; (iii) buccal swab samples from all the personnel involved in the study (archaeologists, anthropologists and laboratory researchers) were also collected to monitor for potential sources of contamination.

Ancient DNA procedures

DNA extraction of the 14 Punic samples (Supplementary Figure S1) and PCR set-up were performed in physically isolated work areas dedicated to low copy number DNA analysis at the ancient DNA laboratory of the Department of Cultural Heritage (DBC), University of Bologna, according to rigorous aDNA laboratory criteria to avoid contamination (Cooper and Poinar 2000; Champlot et al. 2010; Fulton 2012; Knapp et al. 2012, 2015; Fulton and Shapiro 2019). Suitable disposable clothing was worn, and sterile materials were used at each step of the work. Benches and equipment surfaces were regularly cleaned (5% NaClO and 96% Ethanol) and UV irradiated ($\lambda = 245$ nm) to destroy contaminant DNA. Blank extractions and negative controls were included in the entire workflow process to exclude the presence of present-day DNA contamination and the genetic profiles obtained from the ancient specimens were compared with the sequences of the researchers involved in this study to make sure of the absence of exogenous contamination. Finally, PCR run and post-PCR procedures were carried out in a physically separated building at the Laboratory of Molecular Anthropology and Centre for Genome Biology of the Department of Biological, Geological and Environmental Sciences (BiGeA), University of Bologna.

Cleaning and powdering

Ancient samples were decontaminated by removing the outer surface through sterile blades or by a diamond pointy drill-bit with a Dremel® drill (Dremel, Racine, WI, USA). Additionally, the surface of the teeth was gently wiped with 5% sodium hypochlorite (NaClO) and rinsed with nuclease-free water. Bone and tooth samples were then UV-irradiated (254 nm wavelength, 12 V and a distance of 5 cm from the UV source) in a laminar flow cabinet for 60 min from each side, and subsequently ground to a fine powder and stored at 5 °C until use. In par-ticular, teeth were cut transversally at the cementum-enamel junction before sampling dentine powder with a diamond drill bit, set at low speed to avoid heating. While from the bones, the denser inner part was taken with a Dremel® drill and was ground into a fine powder with a mortar. All metallic tools and materials were thoroughly cleaned with DNA-ExitusPlus™ solu-tion (AppliChem GmbH, Darmstadt, Germany) after use and rinsed with 70% ethanol and UV irradiated for 15 min between every sampling procedure to prevent cross-contamination between samples.

Ancient DNA isolation

Before DNA extraction, the drilled bone material (ranging from 100 to 300 mg of bone powder) was digested in 1 mL proteinase K (0.25 mg/mL) and EDTA (0.45 M) lysis buffer, and then the DNA was extracted through a silica-based method (Dabney et al. 2013a), slightly modified as described in Serventi et al. (2018). In brief, after centrifugation for 2 min at maximum speed, the supernatant mixed with 10 mL of binding buffer (5 M guanidine hydrochloride, 40% isopropanol, 0,05% Tween-20, 90 mM sodium acetate and nuclease-free water) was transferred on a Zymo-Spin™ V reservoir (Zymo Research -Irvine, CA, USA) previously treated with bleach and UV light to avoid contamination, and fitted on a MinElute column (Qiagen GmbH, Hilden, Germany). After a centrifugation step, the MinElute column was placed in a col-lection tube and centrifuged at 6000 rpm for 1 min. The col-umn was washed twice with 750 μL of PE buffer (Qiagen

GmbH, Hilden, Germany) and the DNA was eluted in 35 μL of TET buffer (1 mM EDTA pH 8, 10 mM Tris-HCl pH 8, 0.05% Tween-20). To confirm the authenticity of the results, for each ancient sample at least two extractions were undertaken at dif-ferent time points and by different investigators. All extracts were stored at −20 °C in siliconized tubes. The DNA concentra-tion in all final extracts was measured using the Qubit® dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA, USA).

Real-time PCR quantification

Quantification of the collected samples was also carried out with the Quantifiler® Trio DNA Quantification Kit (Thermo Fisher Scientific, Oyster Pint, CA) according to the manufacturer's instructions. Quantitative PCR was performed using the 7500 real-time PCR system (Thermo Fisher Scientific, Oyster Point, CA) with 96-well Optical MicroAMP plates following the manufacturer's instructions. Data were analysed using the HID real-time PCR analysis Software v1.2 according to the default setting provided with the Quantifiler® templates.

Modern DNA sampling and extraction

Saliva samples from unrelated volunteers of the present-day Sardinian populations of Cabras ($N = 47$) and Belvì ($N = 27$)(Figure 1) were collected using the Oragene-DNA Self Collection Kit OG-500 (DNA Genotek, Ottawa, Ontario, Canada). The sampling design was aimed at including one coastal population (Cabras) from the same area of Tharros necropolis, to test the genetic continuity across time in the same territory. We also included another population (Belvì), located not so far away from Tharros, but in the inner part of the island, and thus probably affected by different past population dynamics with respect to the coast. An accurate sampling strategy, based on at least three generations residency criteria and specifically focused on longstanding families rooted in the territory, was performed in the attempt to reduce the effects of possible recent external gene flows. Written informed consent was obtained and signed by each participant prior to the recruit-ment and the study was approved by the Bioethics Committee of the University of Bologna.

The genomic DNA of modern Sardinian samples was purified from the Oragene-DNA collection kits following the manufacturer's recommendations and quantified with the Qubit® dsDNA BR Assay Kit (Life Technologies, Carlsbad, CA, USA).

Analysis of the MtDNA control region

Ancient Punic samples

The first hypervariable segment (HVS-I) of the mtDNA D-loop control region for the ancient Punic samples was amplified

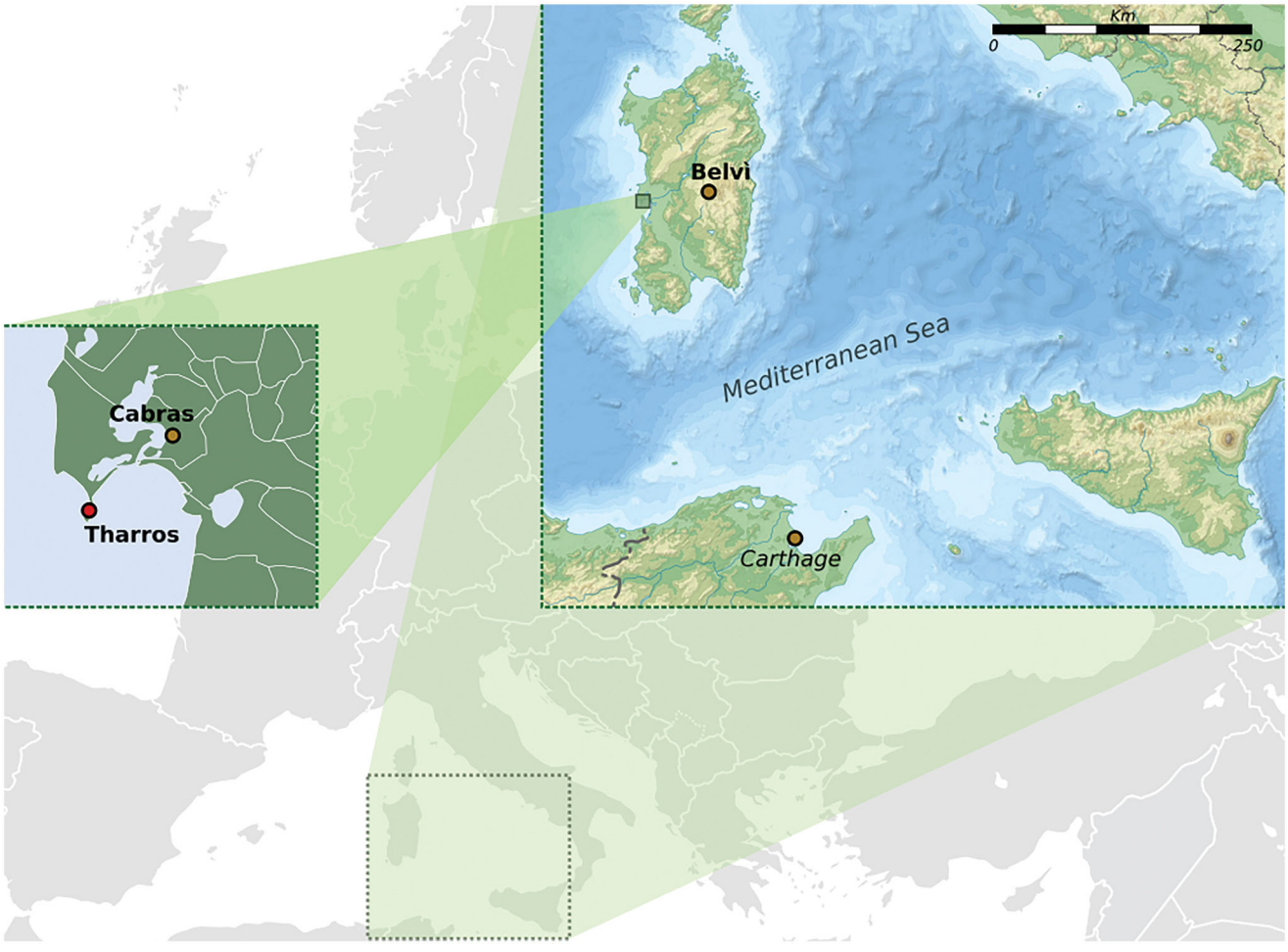

Figure 1. Geographic map showing the approximate geographic locations of the modern Sardinian populations of Cabras and Belvì, as well as of the ancient necropolis of Tharros in the Sinis Peninsula.

Table 1. Haplogroup assignments and HVS haplotypes of ancient and modern samples newly analysed in the present study.

Population	Sample	HVS range	HVS haplotype	SNP in coding region	Haplogroup	*SSH (HVS I motif)
Tharros	TH1[#]	16024 16383	16093C 16212G 16222T 16255A	7028C	H	
Tharros	TH2[#]	16024 16383	rCRS	7028C	H	
Tharros	TH3	16024 16383				
Tharros	TH5	16024 16383	rCRS		H	
Tharros	TH6	16024 16383	rCRS	7028C	H	
Tharros	TH7[#]	16024 16383	16256T 16270T 16399G	7028C	H	
Tharros	TH8	16024 16383	rCRS		H	
Tharros	TH9[#]	16024 16383	rCRS	3010A	H1	
Tharros	TH10	16024 16383	16093C 16212G 16222T 16255A		H	
Tharros	TH11	16024 16383				
Tharros	TH13	16024 16383				
Tharros	TH14[#]	16024 16383	16129A 16223T 16391A	4529T	I	
Tharros	TH15	16024 16383	16129A 16223T 16391A	4529T	I	
Tharros	TH18	16024 16383				
Belvì	DB4519	16015 16569; 1 131	16311C 16519C	3010A	H1	
Belvì	DB4503	16015 16569; 1 131	16519C	3010A	H1	
Belvì	DB4510	16015 16569; 1 131	16519C	3010A	H1	
Belvì	DB4517	16015 16569; 1 131	16519C	3010A	H1	
Belvì	DB4526	16015 16569; 1 131	16519C	3010A	H1	
Belvì	DB4513	16015 16569; 1 131	16304C	6776C	H3	
Belvì	DB4515	16015 16569; 1 131	16304C	6776C	H3	
Belvì	DB4527	16015 16569; 1 131	16304C	6776C	H3	
Belvì	DB4504	16015 16569; 1 131	16298C 72C		HV0	
Belvì	DB4506	16015 16569; 1 131	16298C 72C		HV0	
Belvì	DB4512	16015 16569; 1 131	16298C 72C		HV0	
Belvì	DB4516	16015 16569; 1 131	16298C 72C		HV0	
Belvì	DB4521	16015 16569; 1 131	16298C 72C		HV0	
Belvì	DB4525	16015 16569; 1 131	16298C 72C		HV0	
Belvì	DB4522	16015 16569; 1 131	16093C 16298C 72C		HV0	
Belvì	DB4518	16015 16569; 1 131	16069T 16126C 16519C 73G		J	
Belvì	DB4502	16015 16569; 1 131	16065A 16069T 16126C 16145A 16231C 16261T 73G		J2a1a1d1	
Belvì	DB4509	16015 16569; 1 131	16093C 16224C 16311C 16519C 73G	10398G!	K1a	
Belvì	DB4523	16015 16569; 1 131	16126C 16230G 16362C 16497G 60.1T 64T	12705C	R0a	
Belvì	DB4514	16015 16569; 1 131	16126C 16294T 16304C 16519C 73G		T2b	
Belvì	DB4505	16015 16569; 1 131	16145A 16182C 16183C 16189C 16249C 73G		U1a1c1c1	*(16145)
Belvì	DB4508	16015 16569; 1 131	16145A 16182C 16183C 16189C 16249C 73G		U1a1c1c1	*(16145)
Belvì	DB4528	16015 16569; 1 131	16145A 16182C 16183C 16189C 16249C 73G		U1a1c1c1	*(16145)
Belvì	DB4507	16015 16569; 1 131	16074G 16189C 16192T 16270T 16362C 73G		U5b1b1g1	
Belvì	DB4524	16015 16569; 1 131	16074G 16189C 16192T 16270T 16362C 73G		U5b1b1g1	
Belvì	DB4511	16015 16569; 1 131	16169A 16192T 16235G 16270T 16519C 73G		U5b3a1a	*(16304!)
Belvì	DB4520	16015 16569; 1 131	16169A 16235G 16270T 16519C 73G	12308G	U5b3a1a	*(16304!)
Cabras	DB4588	16015 16569; 1 131	16519C	7028C	H	
Cabras	DB4553	16015 16569; 1 131	16220C 16274A 16292T 16318G 16519C	7028C	H	
Cabras	DB4537	16015 16569; 1 131	16519C 73G	3010A	H1	
Cabras	DB4531	16015 16569; 1 131	16311C 16519C	3010A	H1	
Cabras	DB4536	16015 16569; 1 131	16519C	3010A	H1	
Cabras	DB4585	16015 16569; 1 131	16519C	3010A	H1	
Cabras	DB4597	16015 16569; 1 131	16042A 16356C 16362C 16519C		H1b6	*(16042)
Cabras	DB4533	16015 16569; 1 131	16157C 16183C 16189C 16519C	6776C	H3	
Cabras	DB4546	16015 16569; 1 131	16157C 16183C 16189C 16519C	6776C	H3	
Cabras	DB4549	16015 16569; 1 131	16157C 16183C 16189C 16519C	6776C	H3	
Cabras	DB4587	16015 16569; 1 131	16157C 16183C 16189C 16519C	6776C	H3	
Cabras	DB4548	16015 16569; 1 131	16519C 93G 95T	6776C	H3	
Cabras	DB4583	16015 16569; 1 131	16519C 93G	6776C	H3	
Cabras	DB4592	16015 16569; 1 131	16288C 16311C 16362C 114T	6776C	H3	
Cabras	DB4534	16015 16569; 1 131	16390A 16519C	6776C	H3	
Cabras	DB4581	16015 16569; 1 131	16051G 16257T 16519C	6776C	H3bb1	
Cabras	DB4530	16015 16569; 1 131	16257T 16299G 16519C	6776C	H3bb1	
Cabras	DB4541	16015 16569; 1 131	rCRS	3992T	H4	
Cabras	DB4596	16015 16569; 1 131	rCRS	3992T	H4	
Cabras	DB4540	16015 16569; 1 131	16069T 16126C 73G		J	
Cabras	DB4555	16015 16569; 1 131	16069T 16126C 16193T 16278T 16327T 73G		J2b1a	
Cabras	DB4580	16015 16569; 1 131	16069T 16126C 16193T 16278T 16311C 73G		J2b1a5	*(16311)
Cabras	DB4594	16015 16569; 1 131	16126C 16292T 16294T 16296T 16324C 16519C 73G		T2a1b1a2	
Cabras	DB4554	16015 16569; 1 131	16126C 16294T 16296T 16304C 16519C 73G		T2b	
Cabras	DB4529	16015 16569; 1 131	16126C 16292T 16294T 16296T 16304C 16519C 73G		T2b3a	
Cabras	DB4532	16015 16569; 1 131	16126C 16292T 16294T 16296T 16304C 16519C 73G		T2b3a	
Cabras	DB4539	16015 16569; 1 131	16126C 16292T 16294T 16296T 16304C 16519C 73G		T2b3a	
Cabras	DB4544	16015 16569; 1 131	16126C 16292T 16294T 16296T 16304C 16519C 73G		T2b3a	
Cabras	DB4550	16015 16569; 1 131	16126C 16292T 16294T 16296T 16304C 16519C 73G		T2b3a	
Cabras	DB4552	16015 16569; 1 131	16126C 16292T 16294T 16296T 16304C 16519C 73G		T2b3a	
Cabras	DB4598	16015 16569; 1 131	16126C 16292T 16294T 16296T 16304C 16519C 73G		T2b3a	
Cabras	DB4593	16015 16569; 1 131	16126C 16292T 16294T 16296T 16304C 16519C 73G		T2b3a	

(*continued*)

Table 1. Continued.

Population	Sample	HVS range	HVS haplotype	SNP in coding region	Haplogroup	*SSH (HVS I motif)
Cabras	DB4579	16015 16569; 1 131	16126C 16292T 16294T 16296T 16304C 16519C 55.1T 57C 59C		T2b3a1	
Cabras	DB4582	16015 16569; 1 131	16126C 16292T 16294T 16296T 16304C 16519C 55.1T 57C 59C 73G		T2b3a1	
Cabras	DB4589	16015 16569; 1 131	16126C 16292T 16294T 16296T 16304C 16519C 55.1T 57C 59C 73G		T2b3a1	
Cabras	DB4584	16015 16569; 1 131	16126C 16292T 16294T 16296T 16304C 16519C 55.1T 57C 59C 73G		T2b3a1	
Cabras	DB4543	16015 16569; 1 131	16051G 16129C 16179T 16182C 16183C 16189C 16362C 16519C 73G		U2e U2e	
Cabras	DB4590	16015 16569; 1 131	16051G 16129C 16179T 16182C 16183C 16189C 16362C 16519C 73G		U5a2	
Cabras	DB4591	16015 16569; 1 131	16192T 16256T 16270T 16311C 16526A 73G		U5a2	
Cabras	DB4586	16015 16569; 1 131	16192T 16256T 16270T 16311C 16526A 73G		U5b3a1a	
Cabras	DB4535	16015 16569; 1 131	16169A 16192T 16235G 16270T 16519C 73G		U5b3a1a	*(16304!)
Cabras	DB4542	16015 16569; 1 131	16169A 16192T 16235G 16270T 16519C 73G		U5b3a1a	*(16304!)
Cabras	DB4545	16015 16569; 1 131	16169A 16192T 16235G 16270T 16519C 73G		U5b3a1a	*(16304!)
Cabras	DB4547	16015 16569; 1 131	16169A 16192T 16235G 16270T 16519C 73G		U5b3a1a	*(16304!)
Cabras	DB4551	16015 16569; 1 131	16169A 16192T 16235G 16270T 16519C 73G			*(16304!)
Cabras	DB4595	16015 16569; 1 131	16182C 16183C 16189C 16223T 16278T 16390A 16519C 73G	10398A 10873T	X2	
Cabras	DB4538	16015 16569; 1 131	16189C 16223T 16278T 16519C 73G	10398A 10873T	X2	

*Sardinian specific Haplogroups as defined by Olivieri et al. (2017) and distinguishing mutational motifs (between parentheses) at the HVS I control region. #Indicates the samples conservatively used for genetic population analyses (see "genetic results and Kinship assessment for ancient samples" section for the criteria used for the selection of these samples). Abbreviation: (amplification failed).

through PCR by using three (L15995-H16132, L16107-H16261, L16247-H16402) overlapping fragments (Caramelli et al. 2003), in order to obtain 360 bp spanning from nucleotide position (np) 16024 to 16383. The amplification of each fragment was performed twice in independent PCR reactions and both strands of the DNA were sequenced in order to verify the reproducibility of the results (Ottoni et al. 2011; Hervella et al. 2015; Lorkiewicz et al. 2015). Furthermore, for each extract a larger fragment of ~400 bp was also amplified with the L15996-H16401 primers pair (Vigilant et al. 1989) to exclude possible contaminations, given that aDNA molecules are often fragmented into shorter pieces, typically ranging between 60 and 150 bp (Prufer et al. 2010).

DNA analysis of the researchers

The mDNA HVS-I sequences of the researchers who handled the ancient specimens during the archaeological excavation and aDNA procedures were determined to make sure of the absence of modern contamination as described in Serventi et al. (2017).

Typing of modern Sardinian samples

Variation at the mtDNA HVS-I and HVS-II regions of the mod-ern Sardinian samples from Cabras and Belvì was investi-gated by sequencing a total of 750 base pairs (bp) encompassing nucleotide positions from 15975 to 155. Polymerase chain reaction (PCR) of the HVSI/II regions was carried out in a T-Gradient Thermocycler (Whatman Biometra, Gottingen, Germany) with the following program of amplification: initial denaturation at 95 °C for 5 min, 35 cycles of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 5 min and a final extension at 72 °C for 15 min. PCR products were puri-fied with the ExoSap-IT1 (USB Corporation, Cleveland, OH) and sequenced with the Big-Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) on an ABI Prism 3730 Genetic Analyser using the facilities available at the Unit of Medical Genetics of the Azienda Ospedaliero Universitaria, Policlinico Sant'Orsola-Malpighi of Bologna. To reduce ambiguities in sequence determination, the forward and reverse primers were used to sequence both strands of HVS-I and HVS-II regions.

Haplotype motifs and Haplogroups assignment

Obtained sequencing data were aligned by using DNA Alignment software (http://www.fluxusengineering.com/align.htm) and compared to the revised Cambridge Reference Sequence (rCRS, GenBank Accession Number NC 012920) (Anderson et al. 1981; Andrews et al. 1999) to define the HVS mutational motifs. The types and frequency of nucleotide varia-tions among the ancient sequences were also checked, such as C→T transitions, which represent the prevalent signal of post-mortem miscoding lesions in authentic aDNA samples (Stiller et al. 2009; Bollongino et al. 2013; Dabney et al. 2013b).

Sequences were then assigned into mtDNA haplogroups based on the diagnostic sites of the D-loop region using Haplogrep2 software (Kloss-Brandstätter et al. 2011) and according to the PhyloTree mtDNA phylogeny built 17 (www.phylotree.org) (Van Oven and Kayser 2009). In addition, 22 hap-logroup-diagnostic SNPs on the mtDNA coding region (4216 L, 4529 L, 4580 L, 7028 L, 10398 L, 10400 L, 10873 L, 12308 L, 12705 L, 14766 L, 3010 L, 3915H, 3936H, 3992 L, 4310 L, 4745 L, 4336 L, 4769H, 4793H, 6776H, 13708 L, 13759 L) were also typed to confirm the assignment preliminarily inferred with the HVS haplotype motifs (Richards et al. 2000; Herrnstadt et al. 2002). The SNPs genotyping was performed by means of two differ-ent multiplex-PCR reactions followed by a single-base extension assay, carried out with the SnaPshot© Multiplex Kit (Applied BioSystems, Foster City, USA) as described previously (Bertoncini et al. 2011). Capillary electrophoresis reactions were performed on an ABI PRISM™ 3130 DNA Genetic Analyser (Applied BioSystems, Foster City, USA) at the Department of Diagnostic and Laboratory Services and Legal Medicine, of the University of Modena and Reggio Emilia.

Comparison populations and statistical analyses

In order to test spatial patterns of genetic variation between ancient Punic and present-day Sardinian samples with

respect to the modern Euro-Mediterranean genetic landscape, a large set of 5590 mtDNA sequences from Europe ($n = 3481$ – Cyprus, Greece, France, Portugal, Spain, Sicily and Sardinia), North Africa ($n = 1597$ – Libya, Morocco, Algeria and Tunisia) and the Near East ($n = 912$ – Lebanon, Syria, Jordan and Palestine) were collected from the literature (Supplementary Table S2). In addition, to provide a temporal overview and directly assess the genetic relationships between past and present-day populations that occupied the same territory at different time frames, a specific set of 127 ancient Sardinian mtDNA sequences, time-wise spanning from the Mesolithic to the Medieval period, were also retrieved from available literature data (Supplementary Table S3).

Standard diversity indexes and pairwise Fst genetic distances were computed on the common 360 bp of the HVS-I sequences by using the Kimura 2P option (Kimura 1980) and a gamma value of 0.26, as implemented in Arlequin software v.3.5 (Excoffier and Lischer 2010). Linearised Slatkin Fst values (Slatkin 1995) were then exploited to reconstruct a non-metric Multidimensional Scaling (MDS), using the R software package "*MASS*" (Venables and Ripley 2002; R-Development Core Team 2011).

In order to better clarify inter-population genetic affinities with respect to either the modern populations included in the Euro-Mediterranean dataset or the ancient Sardinian compari-son groups, the genetic variation at the HVS-I sequences or in haplogroup distribution respectively, was directly assessed through a Principal Component Analysis (PCA) performed with the R software package "*adegenet*" (Jombart 2008). Furthermore, a Median-Joining Network analysis was applied to Cabras, Belvì, the Punic samples from Tharros and available ancient Sardinian mtDNA sequences using the Network 10.1 program (http://www.fluxus-technology.com). Finally, discrimin-ant analysis of principal components (DAPC) was carried out to further investigate the patterns of clustering among the consid-ered modern and ancient Sardinian samples, by using the func-tions implemented in the R software "*adegenet*" package (Jombart et al. 2010).

Results

Authenticity of aDNA results

The strict criteria and the rigorous procedures used in this research (Sampietro et al. 2006; Pruvost et al. 2007; Pilli et al. 2013; Llamas et al. 2017), from the archaeological sampling up to the laboratory analyses, allowed us to exclude present-day DNA contamination and to attest the authenticity of data obtained for our ancient specimens. In particular, no contamination was ever observed in either the blank extractions or in the amplification negative controls, and we considered aDNA profiles as authentic only when a clear sequence was reproduced in all the overlapping portions of each adjacent fragment. Furthermore, even if the archaeological situation recorded in Tharros did not make it possible to take more than one sample from the same individual since the human specimens collected were not in anatomical association with any other part of the individuals' skeleton, nevertheless, during the sample preparation steps all the skeletal remains were divided and stored in two different aliquots and analysed in different experimental lots at different times in order to replicate molecular analyses independently. Accordingly, the HVS-I sequences were considered authentic exclusively when they showed the same haplotype motifs in all the different replicates.

The phylogenetic consistency of obtained haplotypes and matching haplogroup assignments of both HVS-I data and coding region SNPs were indicative of the robustness of the mtDNA genotyping approach presented here. Accordingly, the obtained HVS-I mtDNA sequences made phylogenetic sense and reflected polymorphisms congruent with the geographic location under study. Furthermore, the haplogroup and sub-haplogroup motifs were fully represented and no sequences showed obvious conflict with haplogroup-defining segregating sites.

Finally, all the HVS-I sequences obtained from the Punic samples of Tharros showed different haplotypes from those of the researchers involved in the study and none of the ancient samples screened with the L15996-H16401 primers pair yielded any amplification products, thus indicating the absence of intact modern exogenous DNA.

Real-time quantification results

Real-time quantification analysis was useful to identify both the state of conservation and the sex of analysed samples. Although the reaction failed for some specimens (TH3, TH5, TH10, TH11, TH13 and TH18), presumably due to the low amount of genetic material associated with the high DNA degradation, successful quantification results were however obtained for eight out of the 14 considered samples (Supplementary Table S4). In most of the extracts, only the small autosomal (SA) target was amplified, confirming a high degradation of the DNA molecules. Furthermore, since for many of the analysed samples no results were obtained by the large autosomal (LA) target, the degradation index (DI) was evaluated for only two specimens (TH1 and TH7), indicating the presence of significant degradation of the extracted genetic material and thus suggesting the ancient origin of the two DNA templates. The Quantifiler® Trio kit also allowed the calculation of the ratio of total autosomal DNA with respect to the male-specific Y-chromosome DNA (M:F), a measure which is particularly useful to assess mixture samples and to check for possible contamination, that does not result among the outcomes obtained for our ancient samples (Supplementary Table S4).

Genetic results and kinship assessment for ancient samples

HVS-I mitochondrial consensus sequences were successfully obtained for 10 out of the 14 analysed ancient Punic samples (Table 1) representing an overall success rate of 71.43%. The remaining 4 samples were excluded from subsequent analyses because they yielded no mtDNA amplification products (TH3 and TH18) or produced ambiguous sequence

results (TH11 and TH13). The genotyping of the 22 coding region SNPs by means of multiplex amplification failed for three (TH5, TH8 and TH10) out of the ten above-mentioned samples.

By combining both HVS-I sequencing and whenever possible the SNPs genotyping results, overall the analysed Punic samples were classified as belonging to the mtDNA macro-haplogroups H and I (Table 1). Importantly, even if for some cases it was not possible to confirm the haplogroup assignment due to the failure of SNPs genotyping (Table 1), some samples belonging to the same graves also showed the same mutation motifs; in particular: i) TH1 (S.U. 178, male, long bone) and TH10 (S.U. 178, femur LS) from the grave A2; ii) TH2 (S.U. 178, male, long bone), TH5 (S.U. 188, femur) and TH6 (S.U. 188, male, femur) from the grave A2; iii) TH8 (S.U. 178, female, femur LS) and TH9 (S.U. 178, female, femur RS) from the grave A2; iv) TH14 (S.U. 167, the male, tooth) and TH15 (S.U. 167, the male, tooth) from the grave Z). Nevertheless, since the collected ancient Punic samples from the necropolis of Tharros were not found to be associated with any other part of the human skeleton, it was not possible to establish the exact number of individuals buried in the A2 and Z graves and therefore to assess whether two or more samples within the analysed set actually belonged to the same individual. In addition, the fact that some individuals buried in the same grave could be maternally related might introduce a further bias in the statistical analyses of genetic data. For these reasons, and in conjunction with the results of the Real-Time analysis (sex determination) and with the type of bone analysed, the haplotypes observed multiple times in the same grave were considered only once in the subsequent analyses, with the hypothesis that they could have originated from the same or related individuals. We thus conservatively considered only five haplotypes for the downstream analyses (Table 1).

Genetic composition of modern Sardinian samples

The 74 samples from the two present-day Sardinian populations of Cabras and Belvì were assigned to 24 different mtDNA genetic lineages (including sub-lineages), overall belonging to the R, H, HV, J, K, T, U and X macro-hap-logroups (Table 1). In accordance with the typical patterns of mtDNA genetic variability documented for Western Europe and the Mediterranean, most of the analysed individuals belonged to the super-haplogroup H (and particularly to H1 and H3 sub-haplogroups), which on the whole accounted for the ∼30% and 40% of the mtDNA lineages detected in Belvì and Cabras, respectively. The second most frequent lin-eage in Belvì was represented by the haplogroup HV0, which is present in 7 individuals, thus corresponding to ∼26% of the variability. On the other hand, a further amount of ∼30%of mtDNA genetic lineages in Cabras was accounted for by the T2 haplogroup, and particularly by the T2b3a sub-lineage present in 12 individuals. The next most frequent hap-logroups were U and J sub-lineages, which overall repre-sented 26% and 7% of the variability in Belvì, and the 19% and 6% in Cabras, respectively. The other remaining lineages instead showed frequencies lower than 5%.

Interestingly, some individuals from both Belvì and Cabras were found to belong to the Sardinian-Specific Haplogroups (SSHs) H1b6, J2b1a5, U1a1c1c1 and U5b3a1a defined by Olivieri et al. (2017), also showing the distinguishing muta-tional motifs at the HVS-I control region (Table 1). In particu-lar, the SSHs detected among Cabras and Belvì individuals were estimated to coalesce mainly in Neolithic–Copper Age (4–7.8 Kya) or Nuragic (2–4 Kya) periods (Olivieri et al. 2017), suggesting lineages that may have been already present on the island with respect to subsequent post-Nuragic (<2 Kya) events.

Comparison with the modern Euro-Mediterranean genetic landscape

In order to set the observed mtDNA genetic variation into a wider Euro-Mediterranean context, the analysed samples from both the ancient Punic necropolis of Tharros and the present-day Sardinian groups of Cabras and Belvì were com-pared with almost 6000 sequences from a wide set of mod-ern European, North-African and Near-Eastern comparison populations extracted from the literature, by performing a PCA analysis on the HVS-I common set of 360 bp. The first principal component (PC1), accounting for 13.24% of overall variability, identified an axis of genetic variation which sepa-rates North-African groups on the right side of the plot, with respect to all modern Sardinian populations that instead cluster on the opposite side of the x-axis (Supplementary Figure S2), revealing that Cabras, to a lesser extent and Belvì, indeed deviate along the first and second dimensions, respectively. Consistent with lower levels of within-popula-tion variability, Cabras and Belvì also showed amongst the lowest values of genetic diversity (0.8890 ± 0.0291 and 0.9202 ± 0.0313, respectively) after the Ogliastra region (0.8720 ± 0.0096), if compared to the mean value (0.9559; range 0.9130–0.9865) observed for the other modern Sardinian populations (Supplementary Table S5). Similarly, possible evidence of higher inbreeding has been signalled as well by the presence of a reduced number of different hap-lotypes even when considering the whole HVS-I and HVS-II typed regions (28/47 for Cabras and 14/27 for Belvì). At the same time, the stringent adopted sampling strategy used for our population sampling should also be taken into account when interpreting the results.

With respect to the ancient Punic samples from Tharros, it is worth noting how they appear genetically closer to North African populations; indeed, rather than clustering with other modern Sardinians, they instead occupy an intermediate position on the right side of the PCA plot between North African groups and Southern European Iberian populations (Figure 2).

Genetic relationships with ancient Sardinians

To better understand the temporal genetic relationships between current and ancient Sardinian populations, and to

explore how and to what extent past events may have influenced the observed maternal genetic variability through time, the Punic samples from Tharros, as well as the modern populations of Cabras and Belvì, were then compared with a wide set of ancient Sardinian mtDNA sequences dated in a period between the Mesolithic and the Middle Ages. The considered ancient samples were particularly grouped into Mesolithic, Neolithic, Chalcolithic/Early Bronze-Age, Nuragic, Iron-Age, Punic and Roman-to-Medieval periods based on their corresponding archaeological phases and estimated ages (Supplementary Table S3).

Phylogenetic relationships among Sardinian samples were firstly explored through a Median-Joining Network analysis (Figure 3). Interestingly, different clades (U5b, T2b, J2, K1 and HV0) encompassing samples from the modern populations of Cabras and Belvì, were found to contain also mainly pre-Phoenician Sardinian individuals from the Neolithic to the Nuragic periods. On the other hand, the macro-clade of H-sublineages largely includes samples from all the different periods - from the Neolithic up to the present, also compris-ing most of the Punic (including those from Tharros) and later ancient Sardinian individuals (Figure 3). The other non-H Punic sample from Tharros instead belonged to the hap-logroup I, which has also been observed in another Sardinia Punic individual reported by Marcus et al. (2020). Importantly, the reduced number of analysed haplotypes from Tharros, as well as the lower availability of ancient DNA sequences from the Punic with respect to the earlier periods, could have at least partially influenced the observed cluster-ing patterns and should therefore be considered when inter-preting the results of the Network analysis, by also considering that this study is limited to the analysis of the only HVS-I variation.

In order to further explore the suggested patterns, we carried out a DAPC analysis with the aim of formally investigat-ing the genetic structuring both within and among the considered Sardinian groups. DAPC results seem to support the patterns found in the Network, identifying overlapping signatures between Cabras and Belvì haplotypes with main pre-Phoenician clusters of populations (i.e. from Neolithic, Chalcolithic/Early Bronze-Age and Nuragic periods), com-pared to the clustering of the samples from Tharros with the other Sardinian Punic individuals along the second dimen-sion (Figure 4).

Genetic relationships between ancient and present-day Sardinian samples were finally also investigated in terms of haplogroup distribution, by means of a PCA analysis based on the frequencies of the main mtDNA detected lineages (Supplementary Table S6). While being aware of the above-mentioned issues concerning the overall sample size and representativeness of certain groups, PCA results seem to resume the temporal patterns of genetic relationships among the considered samples that have also been observed in the other performed analyses. In fact, the PC1 follows a sort of timeline distribution, ranging from the Neolithic, Chalcolithic/Early Bronze-Age and Nuragic groups on the left of the plot to the Punic, Roman-to-Medieval and modern populations on the right side. The second PC instead reflects the patterns also suggested by HVS haplotype-based analyses, with the samples from Tharros projecting towards the other Punic and later groups, with respect to the modern Cabras and Belvì populations and the pre-Phoenicians ancient Sardinian individuals (Supplementary Figure S3).

Discussion

In this study, we analysed the mtDNA genetic composition of ancient Punic samples associated with the southern necropolis of Tharros in the Sinis Peninsula of Sardinia compared to the patterns of genetic variation observed for present-day Sardinian groups (Figure 1), with the aim to understand the local dynamics of interaction and explore the genetic impact of past population events on the composition of maternal genetic variability through time.

Overall, despite some signals of genetic differentiation possibly related to a recent history of higher isolation/inbreeding and drift (Supplementary Figure 1, Supplementary Table S5), the analysed modern populations of both Cabras and Belvì were found to be sited within the broader range of the Sardinian maternal genetic variation, consistently clustering among the other present-day Sardinian groups in the PCA plot (Figure 2). Accordingly, most of the analysed individuals from Belvì and Cabras also belonged to the H1 and H3 haplogroups, which were reported to be the lineages most frequently found among modern Sardinians. In particular, their presence in ancient Sardinian individuals since the Neolithic, comprising both pre-Phoenician and later samples, agrees with the results from previous studies (Olivieri et al. 2017; Matisoo-Smith et al. 2018) that suggested at least a Neolithic – if not an earlier – introduction into the island. At the same time, the presence of Sardinian-specific Haplogroups (SSHs) dated at Neolithic–Copper Age (4–7.8 Kya) or Nuragic (2–4 Kya) peri-ods (Olivieri et al. 2017), and the observation of overlapping signatures between Cabras and Belvì with mainly pre-Phoenician Sardinian groups from the Neolithic to Nuragic time frames (Figures 3 and 4), may reflect the presence of lineages among the present-day individuals of Cabras and Belvì that presumptively characterised the maternal genetic composition of the Sardinian population since Neolithic and post-Neolithic periods. These findings parallel the recently emerged evidence from large genome-wide based studies performed on both modern and ancient samples, which described present-day Sardinians to have best preserved the Neolithic farmer and also hunter-gatherer genetic compo-nents, possibly due to relative genetic isolation at least through to the whole Bronze Age/Nuragic period (Fernandes et al. 2020; Marcus et al. 2020). Then, external gene-flows contributing to new ancestries were found to have subse-quently affected the genomic composition of Sardinian pop-ulations in the post-Nuragic period. However, these later admixtures appeared to derive mainly from Eastern and Northern Mediterranean sources that were suggested to have relatively little Steppe-ancestry, consistent with present-day Sardinians, thus retaining a higher degree of Neolithic heritage (Marcus et al. 2020). The genetic continuity

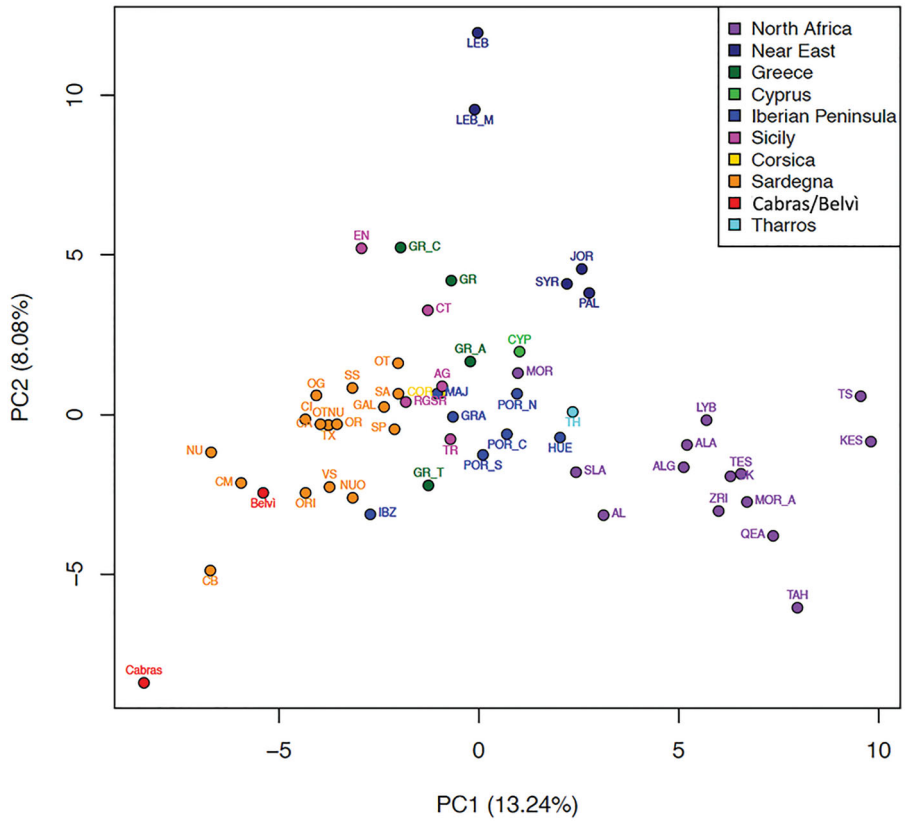

Figure 2. PCA plot showing genetic affinities among ancient Punic samples from Tharros (TH) and present day Sardinian populations of Belvì and Cabras with respect to the modern groups included in the Euro Mediterranean comparison dataset. Populations points are colour coded based on the geographic area of origin as detailed in the legend at the top right of the plot. Modern and ancient samples from the present study are highlighted in red and cyan respectively. Population codes as in Supplementary Table S2.

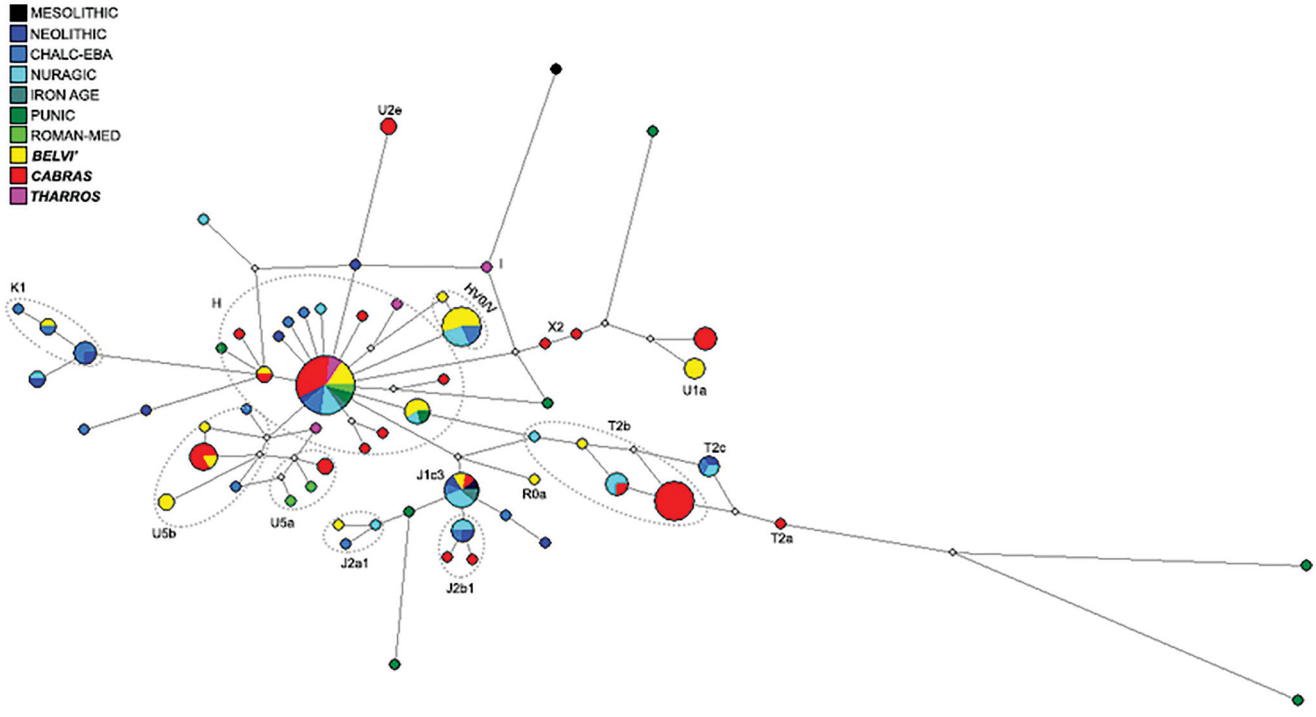

Figure 3. Median Joining Network analysis showing the genetic relationships between available ancient Sardinian sequences and the samples newly analysed in the present study. Circle sizes are proportional to the frequency of a given haplotype and the lines separating haplotypes to the number of mutational steps. Colours show the population group for each of the considered samples as detailed in the legend of the plot.

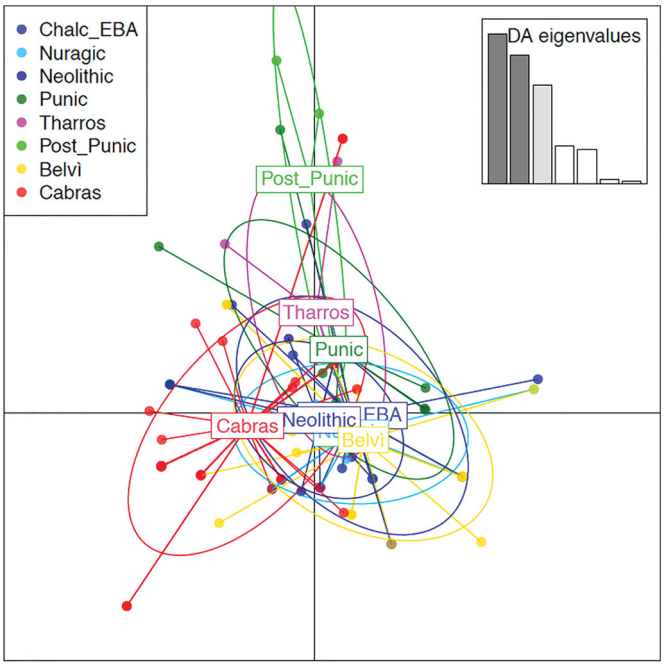

Figure 4. Discriminant Analysis of Principal Components (DAPC) for the mtDNA HVS I haplotypes of ancient Punic samples from Tharros and present day Sardinian populations of Belvì and Cabras with respect to the available ancient Sardinian sequences. Individual haplotypes are represented by points colour coded based on the population groups of each considered sample, as detailed in the legend of the plot. The range variance and the centroid for each popula tion group are highlighted by corresponding ellipses and boxed labels.

observed by these aDNA-based studies between samples from the earlier to the Nuragic periods was found to contrast with the higher heterogeneity observed in the Iron Age and later times (Marcus et al. 2020). Furthermore, a certain substructure has also been detected among present-day individuals, that showed a broader range of variability in ancestry. Importantly, a whole-genome sequencing study on modern Sardinian groups particularly highlighted increased levels of Neolithic farmer (EEF) and Hunter–Gatherers (WHG) components especially in present-day groups coming from more isolated areas - such as the mountainous Ogliastra region, relative to Sardinian populations from less isolated contexts (Chiang et al. 2018). Furthermore, different degrees of post-Nuragic admixture, with a higher "Eastern Mediterranean" ancestry in the southwest (Carbonia, Campidano) and a higher "Northern Mediterranean ancestry" in the northeast (Olbia, Sassari) were also invoked to characterise the fine-scale genetic structure in contemporary Sardinians (Marcus et al. 2020), reflecting known historical events – i.e. major Phoenician and Punic settlements along the south and west coasts, and higher contacts with Italian–Corsican speaking groups, immigrated to the north-eastern part of Sardinia on the other hand.

In this context it is interesting to note that our ancient Punic samples from the necropolis of Tharros specifically departed from the bulk of present-day Sardinian samples towards the North African and Iberian Populations (Supplementary Figure S3). On the other hand, although among our Punic samples we have observed the presence of mtDNA lineages – particularly belonging to the haplogroup H, largely recoded in modern Sardinian populations, the macro-clade of haplogroup H sub-lineages includes samples from all the different periods, indifferently encompassing both pre-Phoenician and later ancient Sardinian individuals. Furthermore, the necessity to be able to rely on a higher number of aDNA complete mitogenomes for interpreting the spatial and temporal patterns of genetic variation within the Haplogroup H and to assign specific subtypes to particular regions or periods has been largely highlighted (Brandt et al. 2015). In this respect, the reduced number of analysed haplotypes from Tharros, as well as the lower availability of ancient DNA sequences from the Phoenician and Punic with respect to the earlier periods, may therefore be taken into account.

The fact that our samples from Tharros can be included in the Punic group already identified in Sardinia is unsurprising, as it perfectly aligns with what is currently known of island archaeology. On the one hand, the proximity to North African and Iberian populations seems very interesting in general, as it confirms the existence of internal and circumscribed migratory phenomena in the central-western basin of the Mediterranean. On the other hand, despite the present indemonstrability of a connection with indigenous Sardinian groups, the quantitative scarcity of the current samples does not allow one to consider these data definitive. Finally, the mitochondrial reference to North Africa appears greatly important since it testifies to female participation in mobility phenomena from North Africa, thus contrasting with the hypothesis that the population colonies were managed only by men who engaged in mixed marriages with native women.

This study offers the first preliminary insights into the population dynamics of the Punic site of Tharros paving the way for future studies. Obviously, the next analyses need to rely on a higher number of samples as well as to take advantage of the analysis of complete genomes, genome-wide autosomal data, or mitogenomes, in order to provide a better understanding of the complex patterns of past population mobility and interaction, overall contributing to further integrate archaeogenetic with archaeological and historical perspectives.

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Disclosure statement

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