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Article *in* American Journal of Physical Anthropology · October 2015

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The Early Colonial Atlantic World: New Insights on the African Diaspora from Isotopic and Ancient DNA Analyses of a Multiethnic 15th–17th Century Burial Population From the Canary Islands, Spain

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KEY WORDS African Diaspora; ancient DNA; stable isotopes; skeletal markers of physical activity; Canary Islands

ABSTRACT

Objectives: The Canary Islands are considered one of the first places where Atlantic slave plantations with labourers of African origin were established, during the 15th century AD. In Gran Canaria (Canary Islands, Spain), a unique cemetery dated to the 15th and 17th centuries was discovered adjacent to an ancient sugar plantation with funerary practices that could be related to enslaved people. In this article, we investigate the origin and possible birthplace of each individual buried in this cemetery, as well as the identity and social status of these people.

Materials and Methods: The sample consists of 14 individuals radiocarbon dated to the 15th and 17th centuries AD. We have employed several methods, including the analysis of ancient human DNA, stable isotopes, and skeletal markers of physical activity.

Results: 1) the funerary practices indicate a set of rituals not previously recorded in the Canary Islands; 2) genetic data show that some people buried in the cemetery could have North–African and sub-Saharan African lineages; 3) isotopic results suggest that some individuals were born outside Gran Canaria; and 4) markers of physical activity show a pattern of labour involving high levels of effort.

Discussion: This set of evidence, along with information from historical sources, suggests that Finca Clavijo was a cemetery for a multiethnic marginalized population that had being likely enslaved. Results also indicate that this population kept practicing non-Christian rituals well into the 17th century. We propose that this was possible because the location of the Canaries, far from mainland Spain and the control of the Spanish Crown, allowed the emergence of a new society with multicultural origins that was more tolerant to foreign rituals and syncretism. Am J Phys Anthropol 000:000–000, 2015.

European colonization of the Americas and the exploitation of its natural resources required a slave population to satisfy the labour shortages and maintain low production costs. African populations were selected to be enslaved because they came from tropical environments and had lower death rates as well as agricultural and technological skills (Goucher et al., 1998). The Atlantic slave trade promoted by Europeans between the 16th and 19th centuries forcibly moved at least 12 million people from Africa to America to be sold mainly as labourers on large plantations in the Caribbean and South America (Frank, 1978; Suret–Canale, 1988; Thomas, 1997; Curtin, 1998; Worger et al., 2010). During the 16th century, this phenomenon was closely connected with the sugar industry, one of the main pillars of the new Atlantic economy (Goucher et al., 1998; Lobo–Cabrera, 1982, 2000).

However, the earliest Atlantic slave plantations were located in the Macaronesian archipelagos, including Madeira, the Canary Islands, and the Cape Verde Islands, at the central–eastern side of the Atlantic Ocean (Curtin, 1998; Thomas, 1997). Textual evidence indicates the existence of sugar cane factories in these regions from the early middle 15th century (Curtin, 1998; Viña, 2014). In this historical and geographical

Grant sponsor: Cabildo de Gran Canaria, Spain; Grant sponsor: Research project HAR2013–41934 funded by MINECO (Spanish Ministry of Economy and Competitiveness).

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Received 16 February 2015; revised 9 September 2015; accepted 21 September 2015

DOI: 10.1002/ajpa.22879
Published online 00 Month 2015 in Wiley Online Library (wileyonlinelibrary.com).

context, the Canary Islands have special importance because they were the only archipelago previously inhabited by pre-European populations, as was the case in the Americas.

The Canarian archipelago was first colonized between the late First Millennium BC and the early First Millennium AD by North African Berber populations (Maca–Meyer et al., 2004). They remained isolated until the 13–14th centuries AD, when Portuguese, Majorcan, and Castilians sailors rediscovered the Islands (Aznar–Vallejo, 2008). After this period of contact and acculturation, Castilians started the conquest of the archipelago in the early 15th century. The native pre-Hispanic population declined quickly due to war, epidemics, and slavery, and the archipelago was completely conquered in 1496 (Tenerife Island), although sugar cane plantations were in operation from the middle of 15th century (Ronquillo, 2008).

Sugar production in the Canary Islands was very important, allowing the accumulation of high revenues and the rise of wealthy individuals in the archipelago (Morales–Lezcano, 1970; Viña et al., 2008). Both the Spanish Crown and private investors made huge efforts to promote the sugar industry after the conquest of the islands. The Crown distributed the new lands to European colonizers, who established sugar cane plantations and built the facilities and factories to process the sugar, an early model for the later colonization of America (Thomas, 1997; Ronquillo, 2008; Viña et al., 2008).

The economy of the Canary Islands during this period was colonial and focused on the export of sugar (Rivero–Suárez, 1991; Viña et al., 2008; Lobo–Cabrera, 2008). The archipelago was also an important Atlantic hub for early maritime trade between Europe, Africa and America, allowing the development of harbors and new cities on the islands. As result, during the 16th century AD the Canary Islands attracted people and investments from Europe, witnessing the first steps in the rise of world capitalism (Morales–Lezcano, 1970; Lobo–Cabrera, 2008).

From the beginnings of production, the Canarian sugar industry had a high demand for laborers (Lobo–Cabrera, 1996, 1998; Ronquillo, 2008). Because of the scarcity of a native population able to work in the plantations, people from Europe and Africa were moved to the islands (Lobo–Cabrera, 1996). Historical texts indicate that in the late 15th and 16th centuries large numbers of people of North and Sub-Saharan African origin were brought to the Canaries to serve as slaves in the sugar industry and as domestic servants for the wealthiest families (Lobo–Cabrera, 1982; Viña, 2006). Acculturation and integration of all of these people into the new colonial society was relatively unproblematic, with more flexible rules than mainland Spain (Lobo–Cabrera, 1982). However, we know very little about this process of acculturation, mostly recorded in historical and administrative documents, with very few archaeological data available (Maca–Meyer et al., 2005; Arnay–de–la–Rosa, 2009). Moreover, the archaeology of the Atlantic slave plantation complex has mostly focused on the Caribbean region, and little attention has been paid to this topic on the Eastern side of the Atlantic.

In this article, we present the results from the study of the human remains uncovered in Finca Clavijo (Gran Canaria, Canary Islands), a recently discovered cemetery that was used between the 15th and 17th centuries AD. Located in an old sugar cane field and far from places where people were ordinarily buried, this is an unusual graveyard that provides unique and new information about an unknown population that could be related to the earliest stages of the transatlantic slave trade. To identify the origin and social status of the people buried at Finca Clavijo we have employed several methods, including the analysis of ancient human DNA, stable isotopes, and skeletal markers of physical activity. Genetic data are very useful as a means of locating the most probable geographical origin of each individual. Oxygen stable isotope analyses are used here to identify migrants and to provide an indication of their likely birthplace. In addition, skeletal markers of physical activity are used to contrast our hypothesis with the level of physical effort documented in the Finca Clavijo population.

MATERIALS AND METHODS

Materials

The cemetery of Finca Clavijo is located in Santa María de Guía, in the northern part of Gran Canaria (Fig. 1). From 1501, the colonial government granted land concessions to be dedicated to the cultivation of sugarcane in this area of the island. These concessions promoted the arrival of employees and slaves (Quintana–Andrés, 2006). The second half of the 16th century saw the rapid development of Santa María de Guía with increases in productive forces, handicraft production, and the need for domestic workers (Macías–Hernández, 2003; Quintana–Andrés, 2006). Finca Clavijo was discovered during building-works, and it covers an area of $\sim$49 m^2. We located seven different burials containing eight individuals in primary positions. In addition, the remains of a further six individuals were found in displaced positions due to the land-works. The actual extension of this necropolis was not completely defined because the archaeological surveys were limited to the area affected by the works. However, surveys conducted with georadar suggest that the cemetery continues around the site (Mendoza–Medina et al., 2010).

The sample from Finca Clavijo cemetery has an MNI of 14. This number was calculated from age, sex and body size, according to Buikstra and Ubelaker (1994), and Outram et al. (2005). Sex and age in adults were estimated by standard protocols (Buikstra and Ubelaker, 1994; Bruzek, 2002; Buckberry and Chamberlain 2002; Schmitt, 2005). For subadults, age was estimated by dental eruption, skeletal maturation, and postcranial measurement of the long bones (Buikstra and Ubelaker, 1994; Schroeder et al., 2009). Sex estimation was not performed in subadults. Age and sex of the individuals are shown in Table 1.

The chronology of Finca Clavijo was determined by two AMS dates and by the nature of the artefacts recovered (Table 1). Individual 2 (Beta–272293) was dated to 460 $\pm$ 40 BP (cal 1410 – 1470 AD). However, in this burial we found a coin with a date of 1559 and a Franciscan medal of the Immaculate Conception, dated to before the 17t^h century (Deagan, 1995, 2002). The sample from individual 7 (Beta–272294) was dated to 420 $\pm$ 40 BP (cal AD 1430–1620). This data set situates the use of this cemetery to the late 15th and early 17th century.

Ancient DNA methods

Authentication criteria for aDNA analysis. Follow-

Following authentication criteria for aDNA work (Cooper and Poinar, 2000; Paabo et al., 2004), strict

measures were taken to avoid contamination. All the pre-PCR procedures were carried out in independent aDNA-dedicated laboratories located at University of La Laguna (Spain). The first step was to decontaminate and powder the human remains. At the second stage, DNA extraction and PCR amplification were carried out. Finally, post-PCR analyses were undertaken in different laboratories.

All personnel involved in aDNA analysis always wore labcoats, faceshields, hats, and gloves. All sample manipulations were performed in laminar flow cabinets, with dedicated pipettes and sterile filter tips. All equipment and work areas were constantly irradiated with UV lamps (Tamariz et al., 2006) and frequently cleaned with bleach (Kemp and Smith, 2005). Certified DNA–free reagents and plastics were commercially acquired whenever possible; otherwise, they were autoclaved and UV–treated. All metallic material was sterilized in an oven at 200°C for at least 4 h.

To facilitate the amplification of the aDNA, we analyzed the mtDNA HVSI region using seven overlapping small fragments. PCR reaction contained a minimum of 1000 – 3000 starting DNA molecules, because lower DNA quantities may be susceptible to artifactual results due to postmortem damage. One blank extraction and three negative PCR controls per extraction and amplification were performed to monitor contamination. As recommended for ancient skeletal remains analysis, positive PCR controls were avoided. Finally, we compared the human remains results with those of an elimination database, containing data from all the researchers involved in the excavation, anthropological studies, and genetic analysis. Modern DNA samples for elimination purposes were processed in a laboratory physically isolated from the aDNA facilities and after all the aDNA analysis had been completed.

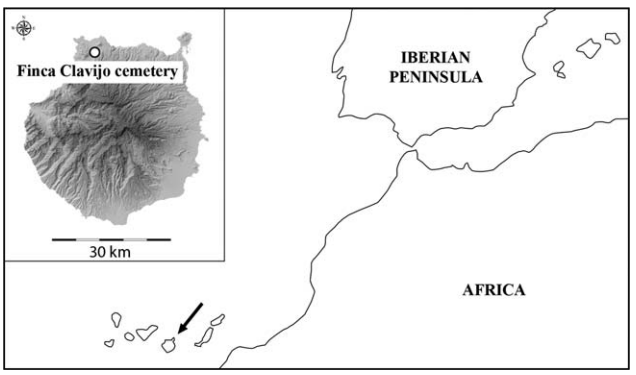

Fig. 1. Map of Gran Canaria Island and Finca Clavijo cemetery.

Samples for aDNA analysis. Thirteen dental remains without fractures, caries or worn areas and one bone were submitted to the aDNA laboratory, numbered from individual 1 to individual 14. Samples for genetic analysis were directly sent to the aDNA laboratory after excavation. They preferably consisted of teeth based on the better conservation of DNA in dental remains. As we only recovered the right leg from individual 8, genetic analyses for this sample were carried out using a phalanx as the source of DNA.

DNA extraction. Tooth samples were washed with 15% HCl, rinsed with UV–treated ddH2O and exposed to UV light for 10 min. To avoid the external surface, teeth were transversely cut through the midline, using an electric dental saw, and the internal pulp and dentine were drilled out using a dental drill. For phalanx sample decontamination, a layer of ~1 mm was removed from the surface using a drilling machine. The bone sample was then drilled using a dental drill.

For DNA isolation, samples were extracted following a modified GuSCN–silica–based protocol (Maca–Meyer et al., 2004). Buccal swabs from all the staff involved in this study were extracted using standard protocols (Rando et al., 1999).

Mitochondrial DNA analysis. Obtaining the whole genome data using next–generation sequencing (NGS) techniques is much more suitable for geographical assignation than focusing on a concrete uniparental locus as mtDNA, especially in potentially admixed population where sex bias is expected. NGS analysis also provides information about damage patterns and fragment length distribution, which are crucial for authenticating aDNA results. For those reasons, NGS and/or whole–genome

TABLE 1. Individual, age, sex, burial position, orientation, date, and grave goods of Finca Clavijo individuals (ND = not determinated)

Individual	Sex	Age	Position	Orientation	Cal. AD	Grave goods and items
1	N.D.	14–16	Right lateral	SW- NE	–	–
2	Female	25–35	Right lateral	W-E	1410–1470	Christian medal/Coin resealed in the year 1559
3	Female	20–25	Right lateral	SW-NE	–	Possible earring/glass beads
4	Female	>45	Left lateral	W-E	–	Earrings
5	Male	35–45	Right lateral	NW-SE	–	Copper shoe lace
6	N.D.	9–12	Left lateral	W-E	–	–
7	Female	20–25	Right lateral	NW-SE	1430–1520 and 1600–1620	Glass beads/earrings/fragment of metal of circular shape
8	N.D.	N.D.	Right lateral	N.D.	–	–
9	N.D.	N.D.	Disturbed	N.D.	–	–
10	N.D.	Subadult	Disturbed	N.D.	–	–
11	N.D.	N.D.	Disturbed	N.D.	–	–
12	N.D.	Subadult	Disturbed	N.D.	–	–
13	N.D.	N.D.	Disturbed	N.D.	–	–
14	N.D.	N.D.	Disturbed	N.D.	–	–

TABLE 2. Isotopic data of Finca Clavijo population

Individual	Context no. (bone/tooth)	Bone sampled	Tooth sampled	$\delta^{18}O_{VPDB}$ (‰)	$\delta^{13}C_{carb}$ (‰)	Carbonate-collagen difference (‰)
1	289/296	Rib	M2	−4.2	−10.7	6.9
2	332/336	L rib	M3	−4.0	−10.5	7.2
3	367/371	L rib	M3	−4.6	−10.9	6.5
4	377/382	L rib	M3	−5.5	−11.2	5.3
5	413/416	L rib	M2	−4.2	−11.1	6.2
6	448/453	L rib	M2	−3.8	−11.0	6.3
7	482/484	R rib	M3	−4.4	−11.1	5.5
8	302	R fibula	n/a	n/a	n/a	n/a
9	168	n/a	M	−4.07	−11.75	n/a
10	167	n/a	M2	−4.44	−11.48	n/a
11	166	n/a	M3	−5.18	−11.67	n/a
12	169	n/a	M	−3.37	−11.09	n/a
13	170	n/a	Premolar	−4.38	−11.85	n/a
14	171	n/a	M2	−4.21	−11.39	n/a

analysis are now preferentially used for aDNA analysis, including slavery studies (Martiniano et al., 2014; Schroeder et al., 2015). As an example, in a recent publication analyzing enslaved Africans remains excavated on the island of Saint Martin, authors were able to link the remains not only to Africa, but also to subcontinental areas within Africa using NGS (Schroeder et al., 2015). However, although the cost NGS is dropping, and the development of capture techniques for endogenous DNA enrichment makes population genetics studies using whole–genome data more affordable (Carpenter et al., 2013), they are still expensive for large sample sizes.

Although in some cases mtDNA is only useful for geographical assignation at a continental scale, and results can be affected by sex–biased admixture, it can provide preliminary evidence about the geographical origin of samples. First, mtDNA is maternally inherited without recombination, so it is helpful to obtain information on the maternal origin of human populations. Second, the high mutation rate of mtDNA, especially in the hypervariable region (HVR), makes it much easier to detect differences between populations. Third, mtDNA has been extensively studied and is well characterized in modern and ancient populations worldwide, including the Canary Islands (both current and indigenous populations), North Africa, and sub-Saharan Africa. Finally, each cell contains many copies of mtDNA so it is more likely that this molecule can be recovered in ancient samples.

Amplifiable mtDNA molecules were quantified using the iQ SYBR Green Supermix kit (BioRad) in a iCycler Thermal Cycler (BioRad). Only those samples with a concentration greater than 100 – 200 copies per microliter were selected for the amplification.

MtDNA HVR I sequencing and RFLP analysis on Finca Clavijo samples were carried out using primer pairs and PCR conditions as previously published (Maca–Meyer et al., 2004). Briefly, we amplified a 400 bp HVRI region (from 16,000 to 16,400) using seven overlapping fragments, with sizes ranging from 82 to 124 bp. In addition, ancient samples were analysed for several SNPs by RFLP analysis, to further characterize haplogroups H, JT, L, and UK. The volume of DNA used in the mtDNA amplification varied depending on its concentration (2 – 10 μl), to incorporate to the PCR reaction mix 1000 – 3000 starting DNA molecules. A 5 μl aliquot of the PCR product was loaded in 10% acrylamide:bis–acrylamide (19:1) gels, stained with ethidium bromide and visualized under UV to assess the amplification yield.

PCR fragments were directly sequenced with both forward and reverse primers used in amplification. Sequencing reactions were performed with the BigDye v3.1 Terminator Cycle Sequencing kit (Applied Biosystems) and run on an ABI PRISM 3130 XL Genetic Analyzer (Applied Biosystems) following manufacturer recommendations. DNA sequences were analyzed using BioEdit software v.7.0.9.0 (Hall, 1999) and haplotypes were obtained by means of HaploSearch software (Fregel and Delgado, 2011), and further confirmed by manually inspecting the electropherograms.

Data analysis. MtDNA sequences were sorted into haplogroups following the most updated classification (mtDNA tree Build 16;(van Oven and Kayser, 2009). Phylogeographic information on mtDNA haplogroups was used to estimate the maternal origin of Finca Clavijo samples (Salas et al. 2004). Published samples from North Africa, Sub-Saharan Africa, and the Canary Islands, as detailed in Supporting Information Table S1, were also used for a more refined haplotypic matches analysis. In that analysis, the geographical origin of each individual was inferred based on the specific regions where its haplotype has been observed. The low sample size prevents any other statistical analysis.

Isotopic analyses

Samples were taken from eight of the primary burials and six samples were taken from the surface finds (although note that these samples cannot be correlated to those individuals analysed for aDNA and are likely to represent less than six individuals). It was not possible to take a sample from individual 8 as only the right leg was recovered. Wherever possible, samples were taken from second or third molars, although these teeth were not available from all of the surface finds (Table 2).

Tooth enamel powder was taken using a hand held drill with a diamond drill attachment. The pretreatment method was based on that described in Balasse et al. (2002); 0.1 ml of 2–3% aqueous sodium hypochlorite was added per mg of sample. The samples were then left for 24 h at 4°C before being rinsed five times with distilled water to remove the sodium hypochlorite; 0.1 mg of acetic acid was then added per mg of sample. The samples were then left for 4 h at room temperature, before the

acetic acid was removed and the samples rinsed. Samples were then frozen and freeze-dried to remove any remaining liquid.

The samples were then transferred to a vial with a screw cap holding a septa and PCTFE washer to make a vacuum seal and dried in an oven at 50°C overnight. They were then reacted with 100% orthophosphoric acid at 90°C using a Micromass Multicarb Sample Preparation System. This produced carbon dioxide which was dried and transferred cryogenically into a PRISM mass spectrometer for isotopic analysis. Carbon and oxygen isotopic ratios were measured on the delta scale, in comparison to the international standard VPDB calibrated using the NBS19 standard (Craig, 1957; Coplen, 1995). Repeated measurements on international and in-house standards show that the analytical error is better than ±0.08‰ for carbon and ±0.10‰ for oxygen.

To compare the results to modern precipitation values, the data must be converted to drinking water values via a series of conversion equations. Multiple equations have been published for each step and there is no consensus on which equation is most appropriate (see Millard and Schroeder, 2010 for further discussion of this topic). Here, we used the equation given in Coplen et al. (1983) to convert the data from carbonate VPDB to carbonate SMOW and the equation given by Iacumin et al. (1996) to convert from carbonate to phosphate. A variety of equations also exist to convert from phosphate to drinking water values: Longinelli (1984); Luz et al. (1984); Levinson et al. (1987); modified Levinson (Chenery et al., 2010); Daux et al. (2008); and Pollard et al. (2011). It is unclear, which equation can be considered the most appropriate, however as Pollard et al. (2011) combines the data from all of the other studies, this equation will be used preferentially. Nevertheless, as each conversion equation increases the error associated with the drinking water value (see Pryor et al., 2014), the data are considered as carbonate VPDB values wherever possible.

To understand and explain the human oxygen isotope results, an estimation of the "local signal" was derived from the closest GNIP international monitoring station at Santa Cruz de Tenerife (Canary Islands). Based on $\delta^{18}O$ measurements of precipitation from 2000 to 2006, the annual mean $\delta^{18}O$ of the precipitation is −1.5‰. Estimations of modern precipitation oxygen isotope values for the site itself, made using www.waterisotopes.org, gives a $\delta^{18}O$ value of −3.0‰ (estimates for latitude 28.1447°, longitude −15.6339°, and altitude 200 m). However, these values are for rainfall. Rainfall in lowland areas of Gran Canaria is very low (<200 mm pa), so it is likely that water was derived from another source, such as wells or springs. Most of the rainfall in Gran Canaria falls in the highland areas, around the mountain Pico de las Nieves which rises to 1949 m in height. The oxygen isotopes of precipitation changes by 0.3‰ with every 100 m increase in altitude, thus the precipitation at the highest point on the island should be depleted by 5.9‰ compared to precipitation at sea level (for the summit of Pico de las Nieves the www.waterisotopes.org estimate of the $\delta^{18}O$ of precipitation is = −6.4‰). The groundwater, and subsequently the wells and springs, is therefore likely have a $\delta^{18}O$ value between −6.4 and −3.0‰.

Skeletal markers of physical activity

Degenerative joint diseases at the peripheral skeleton and spinal column were examined in adult individuals. Its role as a marker of activity lies in the value that is assigned to joint wear as a sign of overstraining of joints, especially in cases where there is asymmetry in the limbs, or focus on specific regions. This disease-marker was diagnosed according to the protocol of Rogers and Waldron (1995). In addition, Schmorl's nodes and herniated discs were recorded on the spinal column (Burke, 2012). These lesions are depressions that occur in the joint surfaces of the vertebral bodies as result of herniation of the intervertebral disk. Some researchers indicated that these conditions are influenced by the biomechanical loads and related trauma. These injures can be generated by an excessive axial load on the spine or by a repetitive stress as result of bending of the axis with a continuous heavy load (Capasso et al., 1999; Burke, 2012). In this regard, several studies have shown that adolescents and young adults with high physical demands have a high incidence of Schmorl's nodes and herniated discs (Ogon et al., 2001; Baranto et al., 2006).

RESULTS

The archaeological evidence

The funerary practices in the Finca Clavijo cemetery were based on primary burial in graves distributed horizontally, without contact between them (Fig. 2). These graves were narrow and designed to contain people in a lateral position. Only one burial contained two individuals, one adult and one subadult, apparently placed simultaneously or after a short period. All bodies were lying on their sides with their heads raised. Individuals 1, 2, 3, 5, and 7 lay on the right side while 4 and 6, 8, did so on the left one. The position of the arms did not follow a strict pattern, appearing stretched or bent on the front or rear of the body. The lower extremities were extended in most cases, with some exceptions where legs were slightly flexed, probably in order to adapt them to the size of the grave. The orientation of the bodies was consistently in a W–E direction. Only the subadult had his head to the East. The direction to which the skulls faced is more heterogeneous, with the exception of burials 4 and 7, which faced South (Table 1).

Individual 2 presents dental alterations on the central incisors of the maxilla. On the right incisor, there is a longitudinal incision on the buccal surface that extends from both sides. In addition, there is an *antemortem* fracture to the medial border. On the left incisor, there is abnormal wear to the mesial edge with a peeling at the end of the labial surface. Macroscopic characteristics of these modifications could be consequence of the use of the teeth as tools or due to artificial manipulations to change the appearance of the teeth (Fig. 3).

There was a varied set of archaeological items inside the graves and in contact with the human remains (Mendoza–Medina et al., 2010). The objects consist of glass beads from bracelets, earrings, a medal, a coin, a shoelace, and a circular fragment of metal (see Table 1). In the grave of individual 2 there was a copper alloy coin of four "*maravedis*." This coin was resealed with a palm leaf stamp by the Cabildo de La Palma (Canary Islands) in 1559 to confirm its value. It derives from a series made in the mint of Santo Domingo during the reign of Charles I prior to the date it was reminted (Gil-Farrés, 1972). In addition, we found interesting items as fragments of sugar molds in other archaeological levels of the site (Mendoza–Medina et al., 2010).

Fig. 2. View of Finca Clavijo cemetery (Author: José Guillén). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

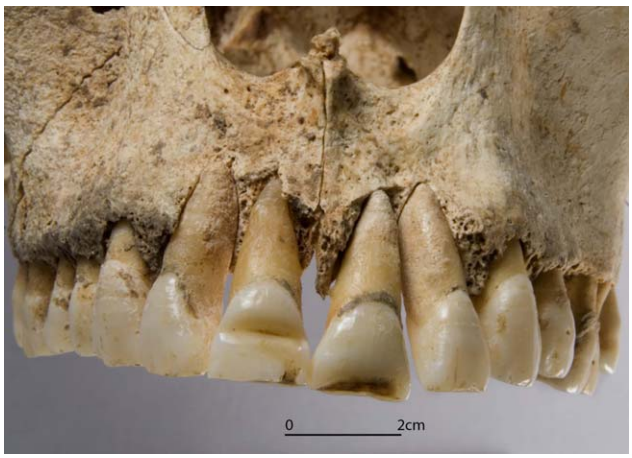

Fig. 3. Dental alteration on the upper incisors in individual 2 (Author: José Guillén). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The mtDNA evidence

The genetic results are consistent with good preservation of human remains at the Finca Clavijo site. All samples analyzed in this study provided enough mtDNA (3000 – 300 copies/μl) to accomplish HVRI sequencing and RFLP analysis. The 14 samples successfully amplified and showed 10 different haplotypes that were classified in eight haplogroups.

The mtDNA haplotypes and haplogroups observed in Finca Clavijo are all different from those in the excavation team and the personnel involved in extracting and analysing aDNA (Supporting Information Table S2), except for three H–CRS samples. Although H–CRS haplotype is frequent in the modern population of the Canary Islands (16%) and some of the putative geographic origins for Finca Clavijo individuals [e.g., 15% in prehispanic Canary Islands (Maca–Meyer et al., 2004; Fregel et al., 2009, 2015), or 17% in Morocco (Ennafaa et al., 2009)], these samples were excluded from subsequent analyses. Future analysis based on NGS will be crucial for authenticating this result.

Two samples from Finca Clavijo (individuals 9 and 13) belong to the H haplogroup (Table 3). Although this lineage is considered of Eurasian origin, it is common in North Africa (Ennafaa et al., 2009). High frequencies of haplogroup H are observed in Berber and Arab speaking Moroccans (≈35%) and drops slightly as one moves southwards to the Sahara and Mauritania (≈20%) (Rando et al., 1998; Gonzalez et al., 2006) and eastwards, reaching <15% in Egypt (Stevanovitch et al., 2004). This haplogroup is also present in aboriginal samples from Tenerife, La Palma, and La Gomera (Maca–Meyer et al., 2004; Fregel et al., 2009, 2015), and in a Gran Canarian prehispanic sample (unpublished data). Two samples (individuals 3 and 10) belong to the R0a haplogroup that displays its highest frequencies in the Arabian Peninsula (Abu–Amero et al., 2008), but is also present in North Africa. Concretely, the haplotype 126 362 has been observed in Morocco (Brakez et al., 2001; Falchi et al., 2006) and Mauritania (Gonzalez et al., 2006). Individual 4 belongs to Eurasian haplogroup I. This haplogroup is also present in Northwest (0.15%) and North Central Africa (1.17%) (Fregel et al., 2009). Individual 2 belongs to the Canarias autochthonous U6b1 lineage (Rando et al., 1998). This haplogroup has a clear Canarian aboriginal adscription, as its distribution is restricted to the Canary Islands. Individual 14 was classified as HV/R and assigned to a Eurasian origin, although more SNP analyses are required to obtain a refined classification. The rest of the samples belong to Sub-Saharan lineages L1b (individual 12), L1c (individual 11) and L2a1 (individuals 1 and 5) pointing to this area as their most probable origin.

Oxygen isotope evidence

As the local baseline value is uncertain and given the difficulties in converting human oxygen isotope values into drinking water values, the oxygen isotope results will first be discussed as unconverted values. The $\delta^{18}O_{VPDB}$ values range from −5.5 to −3.4‰, with a mean of −4.3‰ (standard deviation = 0.6, $n = 13$). As can be seen from Figure 4, there are two clear outliers, Individual 4 and individual 11. These results are also statistical outliers, lying more than 1.5 times the interquartile range below quartile 1. Individual 12 is also identified as a statistical outlier, lying more than 1.5 times the interquartile range above quartile 3.

The $\delta^{18}O_{VPDB}$ results were converted to drinking water values using the equations given in the methodology section, although please note that these converted values have a relatively large error associated with them. The conversion of the $\delta^{18}O_{VPDB}$ values of Individual 4 gives drinking water values between −10.0 and −6.9‰, with a value of −8.1‰ using Pollard et al. (2011). Individual 11 has similar drinking water values to individual 4, between −9.2 and −6.2‰, with a value of −7.5‰ using Pollard et al. (2011). When Unknown 4's $\delta^{18}O_{VPDB}$ result is converted to drinking water values, the results range from −6.2 to −2.2‰, with a value of −4.0‰ obtained using Pollard et al. (2011).

Physical activity markers evidence

Osteoarthritis of the spine was present in three of the five adult individuals (Table 4). The incidence of this disease was considerably higher in individuals 4 and 5 with an incidence of 52% of vertebrae affected for each. However, individuals 3 and 7 were not affected by this

TABLE 3. aDNA result of Finca Clavijo population

Sample ID	RFLP results	HVRI sequence	Haplogroup	Adscription	Matches analysis			
					Modern Canary Islands	Ancient Canary Islands	North Africa	Sub-Saharan Africa
Ind.-09, Ind.-13	7028 *Alu*I -	304	H	Eurasian/ Moorish	X		X	
Ind.-14	7028 *Alu*I +; 12308 *Hinf*I -	278	HV/R	Eurasian/ Moorish	X	X	X	X
Ind.-03, Ind.-10	7028 *Alu*I +; 12308 *Hinf*I -	126 362	R0	Eurasian/ Moorish	X		X	X
Ind.-04	7028 *Alu*I +; 12308 *Hinf*I -	129 148 223 391	I	Eurasian/ Moorish			X	
Ind.-02	7028 *Alu*I +; 12308 *Hinf*I +	163 172 219 311	U6b1	Canarian	X	X		
Ind.-12	7028 *Alu*I +; 12308 *Hinf*I -; 3592 HpaI(+)	126 187 189 223 264 270 278 293 311	L1b	Sub-Saharan African	X		X	X
Ind.-11	7028 *Alu*I +; 12308 *Hinf*I -; 3592 HpaI(+)	129 183C 189 215 223 278 294 311 360	L1c	Sub-Saharan African				X
Ind.-01	7028 *Alu*I +; 12308 *Hinf*I -; 3592 HpaI(+)	223 274 278 294 309 390	L2a1	Sub-Saharan African	X			
Ind.-05	7028 *Alu*I +; 12308 *Hinf*I -; 3592 HpaI(+)	189 192 223 278 293 294 309 390	L2a1	Sub-Saharan African				X

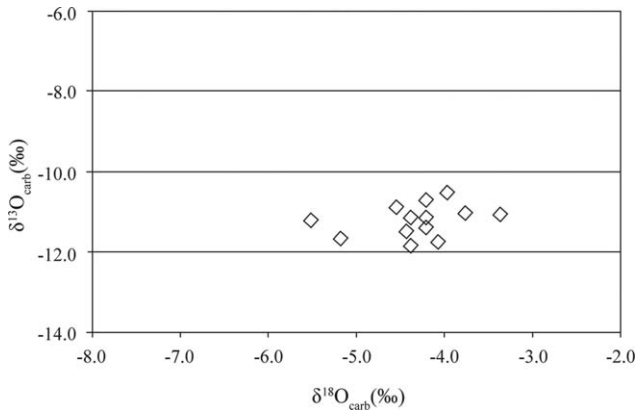

Fig. 4. Carbonate $\delta^{18}O$ and $\delta^{13}C$ results.

condition. Osteoarthritic signals observed were isolated osteophytes of variable size, joint disorders with lipping and several syndesmophytes. This lesion was most important in the C1–C7 (40% incidence) and T10–L5 (20–60% incidence) regions (Fig. 5).

Schmorl's nodes were present in four of five adult individuals (Table 4). This injury was concentrated at the C6, T1, and L5 vertebrae and the region T8–L3 (Fig. 6). It was most important in individuals 3 and 5 with an incidence of 28 and 36%, respectively. Individual 4 was not affected. Herniated discs were only observed in individuals 2 and 5 with an incidence of 20% in each one (Fig. 6). It was concentrated between T6 and T12.

Degenerative joint disease in the form of osteoarthritis was observed on the peripheral skeleton (Fig. 7). This injury was present in the shoulder, knee, and patella with a very low incidence, except in shoulder. Osteoarthirtis in the form of osteophytes, porosities, and lipping was documented in individuals 2, 3, 4, and 5. In addition, eburnation was observed in both shoulders of individual 3.

DISCUSSION

Origin of the people buried at Finca Clavijo

In the late 15th and the first half of the 16th century, people from Africa arrived in Gran Canaria as slaves and they were the most important marginal population in the Canary society of the Ancient Regime (Lobo–Cabrera, 1982, 1996). The Moorish were the largest slave group in the Canaries and came from the neighboring African coast, specifically the area between the south of modern-day Morocco and the Senegal River (Lobo–Cabrera, 1982). Sub-Saharan Africans came to the Canary Islands through several channels: trade agreements with the Arabs; the Portuguese slave trade; and raids carried out by the new colonial Canarian population. Once in the Canary Islands, these people were sold at public auction becoming part of the workforce in houses, convents, and sugar cane factories (Lobo–Cabrera, 1982; Viña et al., 2008). Furthermore, a high number of the indigenous population of the Canaries were captured and sold into slavery during the conquest of the island (Lobo–Cabrera, 1982).

The genetic results showed that the people buried at the Finca Clavijo site had different maternal geographical ascriptions, as is depicted by the phylogeographic analysis of their mtDNA composition. The majority of lineages (H, HV/R, R0a, R*, and I) present in Finca Clavijo, although of Eurasian origin, could have a Northwest African adscription pointing to Moorish slave trade. Other haplogroups (L1b, L1c, and L2a1) have a clear

TABLE 4. Degenerative joint disease on the spine column in adult individuals

	Incidence (%)		
	Osteoarthitis	Schmorl's nodes	Herniated Disc
Individual 2	12	8	20
Individual 3	0	28	0
Individual 4	52	0	0
Individual 5	52	36	20
Individual 7	0	8	0

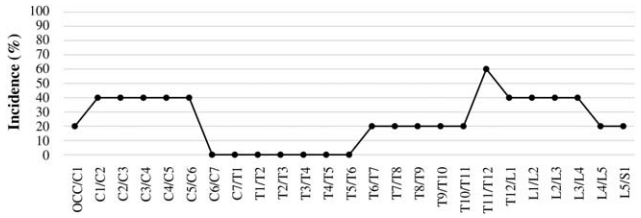

Fig. 5. Osteoarthritis on the vertebral bodies joints in adults.

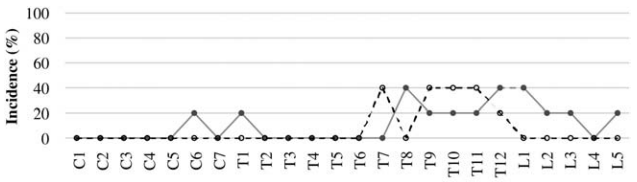

Fig. 6. Schmorl's nodes and herniated discs incidence on the vertebral bodies.

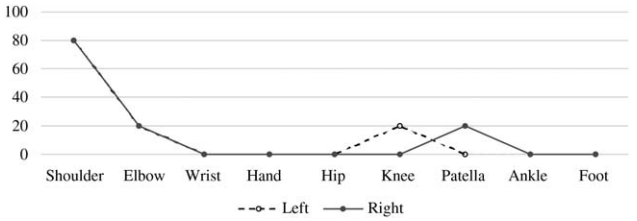

Fig. 7. Osteoarthritis on the peripheral skeleton.

Sub–Saharan African origin. Although some sub-Saharan lineages are present in Northwest Africa due to the gene flow from south to north detected by Rando et al. (1998), matches analysis demonstrates that half of the L haplotypes observed in Finca Clavijo have only exact matches with Sub-Saharan Africa (Table 3). As these types have not been detected in the north, these lineages most probably relate to the black slave trade that occurred in the Canary Islands after the conquest. It is worth mentioning the presence of the Canarian U6b1 haplogroup, signalling the presence of Canarian aboriginal descendants in this cemetery. Moreover, the existence of U6b1 in the sample lets us consider if the other North African lineages belong to autochthonous people or if their presence is due to the Moorish slaves. Attending to the haplotypic matches analysis (Table 3), some lineages present in Finca Clavijo have only been detected in North Africa (and not in the ancient Canarian population). Although difference in sample size could affect to matches estimations, this result could point to a direct gene flow from this area.

Isotope results have contributed in locating two individuals who have lower values than expected for Gran Canaria and therefore may have been born elsewhere (Individual 4 and Individual 11). By comparing their drinking water isotope values (using the Pollard et al., 2011 equation, calculated above) to global maps of the oxygen isotope values of precipitation (www.waterisotopes.org), it is clear that there are several areas around the world where such values can be found. These places include the North African coast and Madagascar, as well as parts of Eurasia and the Americas. Based on the historical and archaeological data, the most likely of these locations as a place of birth for both Individual 4 and Individual 11 is the North African coast. Individual 12 was also identified as a statistical outlier; however, the converted results fall within the range expected for Gran Canaria.

However, the results indicate that the majority of the population drank water from the same source during childhood. The most parsimonious explanation is that these people were born locally, probably on Gran Canaria, although it is also possible that they were all migrants from the same place or places with similar drinking water isotope values. Individuals 11 and 12, while being statistical outliers, have $\delta^{18}O_{VPDB}$ values that lie within two standard deviations of the mean, and thus it is difficult to firmly identify them as migrants. Individual 4, however, can more concretely be said to have migrated during life. The tooth sampled was an M3, and therefore would not have been affected by a breastfeeding signal.

The results of our analysis show that the mtDNA of individuals 11 and 12, L1c and L1b (Table 3), belongs to African lineages. These data match well with the results of the isotopic analyses that suggest a foreign origin for those individuals. However, isotopic analyses are still at an early stage and we cannot get definitive conclusions. This is also the case for individual 4, who belongs to haplogroup I, which is distributed in Europe, Asia, and North Africa. Haplogroup I has not been detected in the pre-Hispanic population of the Canaries, so it is possible that individual 4 came from North Africa. Again, isotopic analyses suggest that individual 4 may be considered a foreigner.

Individual 2 shows sharpening of the front incisors, which could be compatible with extramasticatory tooth-wear using teeth as tools. This female individual belongs to haplogroup U6b1, which is a pre-Hispanic lineage of the Canaries that has not yet been recorded outside of the archipelago. Isotopic analyses also suggest a local origin for this individual. The use of teeth as tools in activities such as leather and fiber work has been recorded in the pre-Hispanic population of Gran Canaria. In all the cases documented, extramasticatory tooth-wear has been observed in a low proportion of females (Delgado–Darias, 2009). Perhaps, the dental wear documented in the individual two was the result of the use of the teeth for this type of work. In this regard, although dental modification was a common practice among the African Diaspora (Stewart, 1939; Stewart and Groome, 1968; Alpers, 2001; Finucane et al., 2008), and it has been recorded in skeletons of slave populations of African origin from colonial

sites of the Caribbean and America (Handler et al., 1982; Price et al., 2006), the type of dental modification practiced by Individual 2 is different.

Identity and ritual at Finca Clavijo

The skeletal markers of physical activity considered in this article suggest that all of the adults buried in Finca Clavijo undertook extensive physical activity that involved significant stress on the spine and appendicular skeleton. Osteoarthritis, Schmorl's nodes and herniated disc values suggest this stress was especially concentred in the lower cervical and lower thoracic vertebrae. As in clinical and archaeological populations, Schmorl's nodes and herniated discs in the Finca Clavijo population are focused primarily in the thoracic regions (Owsley et al., 1987; Faccia and Williams, 2008; Burke, 2012). The data indicate that activities undertaken by the Finca Clavijo population placed particular stress on the shoulder joint. This pattern of use, indicating repeated activity and overemphasis of rotation and adduction, is supported by archaeological and clinical studies (Merbs, 1983; Waldron, 1995; Crubézy et al., 2002; Martin–Dupont et al., 2006; Schrader, 2012). High rates of osteoarthirtis, Schmorls' nodes and herniated disks have been observed in enslaved populations from sugar cane plantation such as Remley Plantation in South Carolina (Rathbun, 1987), Waterloo in Suriname (Khudabux, 1999) and Newton cemetery in Barbados (Shuler, 2005). However, it is difficult to associate these conditions with specific actions, as these markers can be the result of different activities.

The markers reported in the Finca Clavijo series suggest a significant pattern of physical activity, similar to the one described by historical texts for the enslaved population of sugar cane plantations in Gran Canaria. This work included activities in the mills, in the cultivation and harvesting of sugar cane and transportation of products (Brito, 2006). Men performed these tasks, although women also had an important role in other activities that required hard physical effort (Lobo–Cabrera, 1982). In addition, in Santa María de Guía, the nearest village to the Finca Clavijo cemetery, there is textual evidence for the presence of African slaves working in sugar cane plantations or as domestic servants during this period (Andres–Quintana, 2006; Ronquillo, 2008). Here, we suggest that the Finca Clavijo population was probably comprised of individuals who spent their working life on plantations.

The funerary practice reported in Finca Clavijo cemetery is unusual for the Canary Islands during the 15th and 17th centuries, which suggests a differential social status for the people buried in this site. In a historical period where the funeral rituals were performed strictly according to the precepts of the Catholic religion, the presence of unorthodox burials are usually the result of marginalization that certain groups or individuals experienced in life for various reasons, or in other cases, by the manner of death (Ariès, 1987). Examples are deaths that occurred in extraordinary circumstances or for violations of civil and ecclesiastical laws (Lara, 1999; Harding, 2000; Velasco–Vázquez et al., 2003; Di Nola, 2006). This conduct was intended to separate these people from the rest of society, making it public knowledge that certain attitudes or conditions constituted a serious offense that had consequences beyond life. Therefore, it is possible that the people buried at Finca Clavijo were excluded from Christian cemeteries because they were a marginal minority that was not integrated into the civil and ecclesiastical laws. This is unusual even for an enslaved population, since at that time laws in Spain obligated the masters to Christianize their slaves (Lobo–Cabrera, 1982; Phillips, 1990; Arnay–de–la–Rosa and Torres–Palenzuela, 1995; Anaya–Hernández, 2008). Although we can find some examples in America and the Caribbean where enslaved people or African populations were buried in specific places (Hansen and McGowen, 1998; Shuler, 2005).

In this regard, a medal that was buried along individual 2 indicates the use of Christian symbols for this population. The images in the medal show Saint Francis of Assisi on one side, and the Immaculate Conception on the other side. Finca Clavijo cemetery is located near to (less than 1 km) the ruins of the Franciscan convent of Saint Anthony of Padua. This convent was established in 1520 in order to evangelize people in the north–west of Gran Canaria, and it was also devoted to both Saint Francis of Assisi and the Immaculate Conception (Cruz–Saavedra, 2000, 2009). We understand that the medal is an important evidence of syncretism, which indicate that Individual 2 could be a new Christian that was converted in this Franciscan convent.

Some aspects of funerary practices in Finca Clavijo are similar to Islamic funeral ritual, such as the placing the bodies in lateral position (Cara–Barrionuevo, 1993). Literary evidence also indicates the presence of Islamic funerary rituals in the Canary Islands during the 16th century. These practices were banned in 1567, after the "Pragmática Sanción" of Felipe II that prohibited Islamism and Judaism in Spain. In spite of the ban, some African people kept practicing Islamic rituals and burials in the Canaries, because the archipelago was a new frontier with more relaxed rules and laws (Acosta–González, 1989; Anaya–Hernández, 2008, 2013; Fajardo–Spínola, 2005). Consequently, African people could perform Islamic funerary rituals on the islands.

Several important features are unlike Islamic practices, however. Firstly, in Islamic burials the heads should face in the direction of Mecca, which in the case of Gran Canaria, would be located to the east. Only the subadult individual from burial 1 and the individual from burial 3 were found with their heads facing to the east and south-east, respectively. Secondly, in Islamic ritual individuals are placed in the graves in lateral position on the right side and, in most of cases, supine. This position was only found in four of the Finca Clavijo burials, indicating that although the placement of the body on the right side was in the majority, it was not the norm. In addition, we recorded Christian objects in the burials, such as the Franciscan medal, that suggest the occurrence of Catholic rituals. Although we cannot rule out the idea that the Christian items were used to hide or mask the practice of African rituals (Thornton, 1988).

Moreover, we cannot rule out the influence of African customs on the funerary practice of Finca Clavijo because a number of these individuals are of African descent. In Finca Clavijo, there are a number of differently colored glassbeads buried as grave goods, which are very common in the cemeteries of African people in the Americas and the Caribbean (Deagan, 1987; Orser, 1992). These artefacts have been interpreted as originating in Africa or displaying features that can be related to African people (Orser, 1994; Samford, 1996; Handler,

1997). These beads, especially the blue ones, were considered symbols of high status and power in some West-African societies, and show important relationships to Africa (Yentsch and Hunter, 1994; Samford, 1996; Stine et al., 1996; Verhaeghe et al., 2014). African influences, including Islamic, lessened over time due to numerous factors that varied in importance from place to place. Decisive impacts include differences in community cohesion and isolation, changes of settlements from plantations to urban areas, the influence of Christianity (in Spanish territories as a legislative obligation), and the attempt at further integration into the economic and political structures of colonial contexts (Lobo–Cabrera, 1982; Thornton 1992; Jamieson, 1995; Goucher et al., 1998). Furthermore, it is obvious that our limited knowledge of the funerary practices of the African ascendant population in its original environment also influences our imprecise understanding of this topic (Thornton, 1988; Handler, 1997; DeCorse, 1991; Posnansky, 1999; Sweet, 2003; Pearson et al., 2011).

Our interpretation is that Finca Clavijo is a multiethnic cemetery with a singular funerary practice, which is result of the syncretism of various backgrounds such as Catholic, Islamic, and African. Syncretistic funerary rituals were common in the Americas and other places in the Atlantic sphere where African people were involved (Mulago, 1991; Patterson, 1982; Jamieson, 1995; Tishken et al., 2009; Pearson et al., 2011), and are also observed in other spheres such as religion, music, dance, arts, vocabulary, and folklore (Blassingame, 1972; Genovese, 1972; Hall, 1990; Patterson, 1982; Thornton, 1992; Singleton, 1995; Manning, 2014).

Concurrently, the funeral ritual observed in Finca Clavijo can be considered as an indicator of cultural resistance and the capacity for agency of a population that was able to generate procedures of material and symbolic representation of their identity, despite their social marginalization (Ewen, 2000; Groovert, 2000; Kelly, 2008).

CONCLUSIONS

We propose that Finca Clavijo is likely a cemetery where enslaved people were buried. In favor of this interpretation we have several skeletal markers of physical activity that indicate a high level of physical effort, mtDNA lineages related to the geographical places where people were enslaved at that time, and data suggesting that some of these individuals were born far from Gran Canaria. In addition, we observe an unusual burial practice that could be a reflection of different rituals such as African, Islamic, and Christian. Acculturation and integration of slave people into the colonial society in Canary Islands may be a process where social identities had to be reformulated to integrate into an Atlantic society. This cemetery should be understood as the consequence of a very specific situation in the context of the new society that was formulated in the Atlantic colonial world (Goucher et al., 1998; Elliott, 2001; Pietschamnn, 2002). In this regard, Finca Clavijo cemetery provides important archaeological evidence of the early Atlantic world.

ACKNOWLEDGMENTS

The authors express our gratitude to Tibicena SL for permission to study the human remains. Special thanks should be addressed to all the archaeologists who participated in the field work. EL would like to thank James Rolfe for his assistance with isotopic analysis. JM is part of the Research Group IT–622–13/UFI 11/09 of the University of the Basque Country. They wish to thank associate editor and both reviewers for their constructive comments and suggestions.

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