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RESEARCH ARTICLE

# On the origin of Iberomaurusians: new data based on ancient mitochondrial DNA and phylogenetic analysis of Afalou and Taforalt populations

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**ABSTRACT**

The Western North African population was characterized by the presence of Iberomaurusian civilization at the Epiplaeolithic period (around 20,000 years before present (YBP) to 10,000 YBP). The origin of this population is still not clear: they may come from Europe, Near East, sub-Saharan Africa or they could have evolved *in situ* in North Africa. With the aim to contribute to a better knowledge of the settlement of North Africa we analysed the mitochondrial DNA extracted from Iberomaurusian skeletons exhumed from the archaeological site of Afalou (AFA) (15,000–11,000 YBP) in Algeria and from the archaeological site of Taforalt (TAF) (23,000–10,800 YBP) in Morocco. Then, we carried out a phylogenetic analysis relating these Iberomaurusians to 61 current Mediterranean populations.

The genetic structure of TAF and AFA specimens contains only North African and Eurasian maternal lineages. These finding demonstrate the presence of these haplotypes in North Africa from at least 20,000 YBP. The very low contribution of a Sub-Saharan African haplotype in the Iberomaurusian samples is confirmed. We also highlighted the existence of genetic flows between Southern and Northern coast of the Mediterranean.

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Genetic diversity; mitochondrial DNA; ancient DNA; Epiplaeolithic; North Africa

## Introduction

The origin of North African settlement has been investigated by two approaches: First one, focus on the study of the archaeological specimens as well as their environment (Debénath 2000; Aouraghe 2006; Hachi 2006; Mariotti et al. 2009; Belcastro et al. 2010; Barton et al. 2013) whereas the second will compare the molecular diversity of both ancient and current human populations (Stevanovitch et al. 2004; Kefi et al. 2005; Coudray et al. 2009; Ottoni et al. 2009; Ennafaa et al. 2011; Kefi et al. 2015).

Around 20,000 years before present (YBP) to 10,000 YBP, Western North Africa has been occupied by Iberomaurusian populations (Mechtoid), in reference to late Palaeolithic tool industry (Later Stone Age also called Epipalaeolithic) who replaced the Aterian aborigines, in reference to middle Palaeolithic industry (Middle Stone Age). The most important Iberomaurusian sites are Columnata and Afalou (AFA) in Algeria and Taforalt (TAF) in Morocco (Camps 1974; Debénath 2000; Aouraghe 2006; Barton et al. 2013).

The origin of the Iberomaurusians is still a matter of considerable debate (Ferembach 1986; Dutour 1995; Irish 2000; Barton et al. 2013). Anthropological studies have suggested several hypotheses: an European origin (crossing straight from Gibraltar or from Italy via Sicily), a Near Eastern origin, a sub-Saharan African origin or they could have evolved *in situ* in North Africa.

Another debate related to the Iberomaurusian is the question of genetic continuity. Some studies based on morphological features believe that Iberomaurusians have a small contribution to the genetic make-up of later Northwest Africa whereas other studies based on e.g. craniometric, lithic, dental analysis suggest a continuous evolution from Iberomaurusian period to Capsian times (10,000–6000 YBP) (Hachi 1996; Irish 2000; Lubell 2000; Rahmani 2004).

Over the p ast decades, mitochondrial DNA (mtDNA) has become a powerful tool to study human evolution and migration patterns in several geographic areas (Torroni et al. 2006; Melchior et al. 2008; Xu et al. 2008; Green et al. 2010; Haak et al. 2010). It is characterized by a high copy number in cell, maternal inheritance, and a high mutation rate (Taanman 1999; Wallace et al. 1999). It has notably been used to demonstrated the origin of the mitochondrial Eve (Ingman et al. 2000), to highlight the contribution Neanderthal to modern human genome (Green et al. 2008) or to explore the origins of the Denisovans (Meyer et al. 2014).

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Supplemental data for this article can be accessed here.

To study the origin of North African settlements we have chosen to focus on bones exhumed from the area of TAF and AFA. Effectively these bones have been shown to be suitable for DNA analysis as in one hand the protein of these bones presented a good conservation (Audic et al. 1998) and in the other hand, *Beta-globin* gene have been sequenced from one individual in TAF population (Beraud-Colomb et al. 1995).

Previously, we carried out the first molecular study on 31 Iberomaurusian specimens from TAF based on the analysis of the first hypervariable segment of mitochondrial DNA (HVS1 of mtDNA). This study showed that the maternal genetic lineage of TAF population is composed of North African and Eurasian haplogroups. The absence of Sub-Saharan polymorphisms (L0–L7) suggests that the penetration of sub-Saharan mtDNA lineages to North Africa mainly occurred later to Epipalaeolithic period (Kefi et al. 2005; Secher et al. 2014). An individual bearing the 16,223 mutation is present in TAF population but most probably belongs to the U haplogroup than a L lineage according to HaploGrep results (Kloss-Brandstätter et al. 2011).

A recent study based on Radiocarbon dating, determined the age of Iberomaurusian samples of TAF as about 21,000 YBP (Barton et al. 2013). The study, of these ancient samples, should be a way to shade-light the haplogroup datations and should confirm the calculation of coalescence time.

To better understand the origin of North African settlement, we studied the diversity of Iberomaurusian population by analysing the mtDNA of skeletons from the archaeological sites of AFA in Algeria and TAF in Morocco.

We carried out also a phylogenetic analysis of the data relating these Iberomaurusians to 61 current Mediterranean populations.

## Materials and methods

### *Samples/populations*

#### *The population of AFA*

The archaeological site of AFA is a rock shelter located at 30 km from Béjaïa (East of Alger-36°29′N, 3°00′EE) in the North of Algeria (Figure 1). Discovered in 1927, the shelter of AFA was excavated by C. Arambourg from 1928 to 1930. The stratigraphic study highlighted two levels: the upper level of thickness 7.5 m composed of bones and industry, whereas the lower level was shown to be sterile (Arambourg & Boule 1934).

The shelter of AFA is a human ossuary where 50 individuals can be observed. Of these 50 individuals, only eight skeletons fitted for genetic identification (other bones not clearly belong to an individual or another). The excavation of AFA has been started again since 1983, by S. Hachi. The site is dated from 15,000 to 11,000 YBP.

The molecular and the phylogenetic analyses performed in the present study have included seven individuals from the population of AFA (Table 1) preserved in Human Palaeontology Institute of Paris, France. The GenBank accession numbers of these sequences are KR873164 to KR873170.

#### *The population of TAF*

The 'Grotte des pigeons' (34°48′ 38″ N, 2°24′ 30″ W) discovered in 1908, is a cave located at 1 km from the village of TAF and at 55 km from Oujda in Eastern Morocco (Figure 1). During the excavations, carried out by Roche (1944–1976), deposits containing Aterian and typical Iberomaurusian artefacts as well as more than 180 Iberomaurusian skeletons exhumed from 40 burials were described. Further investigations of the cave have been done in the 1980s and a new


**Figure 1.** Geographical localization of Afalou and Taforalt.

excavation phase is actually still in progress (this phase started in 2003).

The Iberomaurusian occupation of the cave was dated between 23,000 YBP and 10,800 YBP (Ferembach 1985). Recently, a high precision radiocarbon chronology made on bones and charcoals showed that the Iberomaurusian industry appeared in TAF at least 22,093–21,420 Cal BP (calibrated YBP) (Barton et al. 2013).

In a previous study, we performed the first molecular analysis of individuals exhumed from the archaeological site of TAF (Kefi et al. 2005). In this study, we solved the problem linked to the nomenclature of TAF's skeletons. The different Arabic numbers, followed the Roman number of the burial, corresponded to different individuals inhumed in the same tomb. This study showed also that the maternal genetic lineage of TAF population is composed of North African haplogroup (U6) and Eurasian haplogroups (H, U, R0). In the present study, we include mtDNA sequences of 23 individuals from TAF population to perform phylogenetic analyses (Table 2). The GenBank accession numbers of these sequences are KR873142 to KR873170.

### Current populations collected from the literature

To perform comparative and phylogenetic analyses, we collected from the literature **4971** mtDNA sequences (nucleotide position 16085–16365) belonging to 61 Mediterranean populations: North Africa ($n = 2052$), the Near East ($n = 231$), and Europe ($n = 2738$) (Table 3).

## Molecular methods

### Laboratory precautions

DNA preserved in bone remains of archaeological specimens, presents specific characteristics, such as limited amount of material as well as degradation (Hofreiter et al. 2001; Kefi et al. 2003; Kefi 2011). Studying such DNA requires drastic conditions and particular approaches, different from those used with modern DNA specimens. We followed the guidelines of authenticity for ancient DNA analysis (Cooper & Poinar 2000; Paabo et al. 2004; Malmström et al. 2007; Bon et al. 2008; Deguilloux et al. 2011). Drastic precautions were taken in order to avoid contamination with

**Table 1.** Haplotypes and haplogroups observed in Afalou individuals.

| Subject | Position | HVS-I haplotype | Haplogroup | Overall rank[a] |
|---|---|---|---|---|
| AF2 | 16054–16317 | CRS | H or U | – |
| AF13 | 16054–16317 | CRS | H or U | – |
| AFXXV | 16054–16317 | CRS | H or U | – |
| AF19 | 16054–16317 | 16126C 16294T 16296T 16304C | T2b | 100% |
| AF22B | 16054–16317 | 16126C | JT or H14b1 | 100% |
| AF3 | 16054–16317 | 16069T 16126C 16128T | J | 100% |
| AF7 | 16054–16317 | 16063C 16069T 16126C | J1c3f | 100% |
| Experimenter 1 | 16054–16400 | 16295T | H103 | 100% |
| Experimenter 2 | 16054–16454 | 16126C 16355T 16362C | R0a1a | 100% |

[a]Score of haplogroup assessment provided by HaploGrep.

**Table 2.** Haplotypes and haplogroups observed in Taforalt individuals.

| Subject | Position | HVS-I haplotype | Haplogroup | Overall rank[a] |
|---|---|---|---|---|
| TafI-21 | 16054–16454 | CRS | H or U | – |
| TafII | 16054–16454 | CRS | H or U | – |
| TafV5 | 16054–16317 | CRS | H or U | – |
| TafV7 | 16081–16404 | CRS | H or U | – |
| TafV20 | 16054–16317 | CRS | H or U | – |
| TafXVa | 16054–16317 | CRS | H or U | – |
| TafXV0 | 16054–16317 | CRS | H or U | – |
| TafXVII | 16054–16317 | CRS | H or U | – |
| TafXIX | 16054–16317 | CRS | H or U | – |
| TafXX-6 | 16054–16317 | CRS | H or U | – |
| Taf55-I | 16054–16454 | 16126C 16355T | R0a1a | 89.67% |
| Taf55-IB | 16105–16317 | 16239T | H1 | 100% |
| TafV18 | 16054–16317 | 16126C 16304C | R0a2c | 100% |
| TafV19E | 16054–16317 | 16172C 16174T | U6d3 | 77% |
| TafV26 | 16054–16317 | 16204C 16226T | H2a2a1 | 50% |
| TafV27 | 16054–16317 | 16298C | H6a1a8 | 100% |
| TafVI-10 | 16054–16317 | 16124C 16239T | H2a1e1a | 78.02% |
| TafVIII | 16054–16317 | 16223T | U4a2b | 100% |
| TafXIXa | 16054–16317 | 16179T 16298C | U4c1 | 76.47% |
| TafXVa2-19 | 16054–16317 | 16189C 16261T | H1 | 84.13% |
| TafXXIV | 16054–16317 | 16126C 16172C 16174T | R0a1a | 77.50% |
| TafXXV3 | 16054–16317 | 16126C | H14b1 | 100% |
| Experimenter 1 | 16054–16400 | 16295T | H103 | 100% |
| Experimenter 2 | 16054–16454 | 16126C 16355T 16362C | R0a1a | 100% |

Molecular investigation was performed in Kefi et al. (2005), haplogroup classification is updated in the present study.
[a]Score of haplogroup assessment provided by HaploGrep.

**Table 3.** Data collected from 61 current Mediterranean populations.

| Populations | Sample size | Code | References |
|---|---|---|---|
| NORTH AFRICA | | | |
| TUNISIA | | | |
| NORTH | | | |
| Qalaat El Andalous | 29 | QAL | (Cherni et al. 2009) |
| Capital Tunis | 98 | CTU | (Cherni et al. 2009; Plaza et al. 2003) |
| El Alia | 48 | ELA | (Cherni et al. 2009) |
| Zriba | 35 | ZRI | (Cherni et al. 2009) |
| Slouguia | 28 | SLO | (Cherni et al. 2009) |
| Testour | 50 | TES | (Cherni et al. 2009) |
| Kesra | 43 | KES | (Cherni et al. 2009) |
| Northern Tunisia | 114 | TUN | (Turchi et al. 2009; Kefi et al. 2015) |
| SOUTH | | | |
| Skira | 20 | SKI | (Cherni et al. 2009) |
| Jerba Arabs | 29 | JEA | (Loueslati et al. 2006) |
| Jerba Berbers | 30 | JEB | (Loueslati et al. 2006) |
| Chenini-Douiret | 53 | CHO | (Fadhlaoui-Zid et al. 2004) |
| Sened | 55 | SEN | (Fadhlaoui-Zid et al. 2004) |
| Bou Omrane | 40 | OMB | (Ennafaa et al. 2011) |
| Bou Saâd | 40 | SAB | (Ennafaa et al. 2011) |
| Matmata | 53 | MAT | (Fadhlaoui-Zid et al. 2004) |
| LIBYA | | | |
| Fezzan (Al Awaynat) | 111 | FAL | (Ottoni et al. 2009) |
| Fezzan (Tahala) | 18 | FTA | (Ottoni et al. 2009) |
| EGYPT | | | |
| Upper Egypt | 102 | EGY | (Stevanovitch et al. 2004) |
| Gurna | 34 | GUR | (Stevanovitch et al. 2004) |
| Siwa Berbers | 78 | SIB | (Coudray et al. 2009) |
| Alexandria | 277 | ALX | (Saunier et al. 2009) |
| ALGERIA | | | |
| Algerians | 47 | ALG | (Plaza et al. 2003) |
| Algerian Mozabites | 85 | MOZ | (Corte-Real et al. 1996; Macaulay et al. 1999) |
| MOROCCO | | | |
| Southern Moroccan (Berbers) | 50 | MBS | (Brakez et al. 2001) |
| Northern Moroccan (Berbers) | 60 | MBN | (Rando et al. 1998; Plaza et al. 2003) |
| Moroccan Arabs | 50 | MOA | (Rando et al. 1998; Plaza et al. 2003) |
| Saharawi | 56 | SAH | (Plaza et al. 2003) |
| Marrakech | 52 | MAR | (Falchi et al. 2006) |
| Asni Berbers | 53 | ASB | (Coudray et al. 2009) |
| Bouhria Berbers | 70 | BOB | (Coudray et al. 2009) |
| Figuig Berbers | 94 | FIB | (Coudray et al. 2009) |
| Total North African sequences | 2002 | | |
| NEAR EAST | | | |
| PALESTINE-ISRAEL | | | |
| Palestinian-Israeli | 117 | PAL | (Richards et al. 2000a) |
| Druze | 45 | DRU | (Macaulay et al. 1999) |
| SYRIA | | | |
| Syrian | 69 | SYR | (Richards et al. 2000a) |
| Total Near Eastern populations | 231 | | |
| EUROPE | | | |
| GREECE | | | |
| Greeks | 184 | GRE | (Villems 2011) |
| Northern Greeks | 319 | GNG | (Irwin et al. 2008) |
| CYPRUS | | | |
| Cypriots | 91 | GRC | (Irwin et al. 2008) |
| TURKEY | | | |
| Turks | 213 | TUR | (Richards et al. 2000a) |
| SPAIN | | | |
| Andalusian | 158 | AND | (Corte-Real et al. 1996; Crespillo et al. 2000; Plaza et al. 2003) |
| Andalusia (Granada Province) | 66 | AGP | (Falchi et al. 2006) |
| Catalan | 162 | CAT | (Plaza et al. 2003; Alvarez-Iglesias et al. 2009) |
| Galician | 374 | GAL | (Salas et al. 1998; Alvarez-Iglesias et al. 2009) |
| Basque | 45 | BAS | (Bertranpetit et al. 1995) |
| Majorcan | 112 | MAJ | (Picornell et al. 2005; Falchi et al. 2006) |
| Minorcan | 46 | MIN | (Picornell et al. 2005) |
| Valencian | 42 | VAL | (Picornell et al. 2005) |
| Ibizan | 50 | IBI | (Picornell et al. 2005) |
| Chuetas | 48 | CHU | (Picornell et al. 2005) |
| FRANCE | | | |
| French | 109 | FRE | (Dubut et al. 2004) |
| Centre Corsica | 47 | COR | (Varesi et al. 2000) |
| Southern Corsica | 53 | COB | (Falchi et al. 2006) |
| ITALIA | | | |
| South Italy | 86 | ITS | (Francalacci et al. 1996; Richards et al. 2000b) |
| Sardinian | 69 | SAR | (Di Rienzo & Wilson 1991) |

(*continued*)

**Table 3.** Continued

| Populations | Sample size | Code | References |
|---|---|---|---|
| Central Sardinia | 51 | SAN | (Falchi et al. 2006) |
| Northern Sardinia | 50 | SNS | (Falchi et al. 2006) |
| Sardinian (Trexenta) | 47 | SAT | (Falchi et al. 2006) |
| Sardinian (San Pietro island) | 44 | SSP | (Falchi et al. 2006) |
| Sardinian (Sant' Antioco island) | 42 | SSA | (Falchi et al. 2006) |
| Sicilian | 169 | SIC | (Richards et al. 2000b; Cali et al. 2001) |
| Tuscan | 61 | TUE | (Falchi et al. 2006) |
| Total Eurasian sequences | 2738 | | |
| Total studied sequences | 4971 | | |

contemporary DNA. The extraction was performed in a 'clean room' located in a laboratory where the study and the amplification of Human DNA had never been done before. Mask, coat, gloves, sterile materials, and dedicated pipettes with aerosol resistant plugged tips were used in all steps of manipulation. The worktop was cleaned with bleach, ethanol, and 'DNA away' (Molecular Bio Product, Inc., San Diego, CA). The DNA extracting solutions were stored in a second room isolated from the others. Before use, all buffer solutions were autoclaved and tested by PCR to make sure of the absence of contamination.

Extractions have been duplicated in two independent laboratories (Faculty of Science, Luminy and Laboratory of Anthropology in the Faculty of Medicine, Marseille, France).

Amplifications were carried out in another building (Sainte Marguerite Hospital, Marseille, France). PCR and Post-PCR manipulations were carried out in separate laboratories. PCR buffers were exposed to UV light prior to use (excluding DNA polymerase). Parallel to each experiment, contamination was estimated using negative controls (reagent without sample).

### *DNA extraction*

Femur fragments were sampled from skeletons of AFA. The bone outer surfaces were removed and cleaned as described in Kefi et al. (2003). Bone was reduced to a powder using electric drill. The powder obtained (0.5 g) was digested for 48 h with proteinase K (0.5 mg/ml) in a buffer [0.5 M ethylene diamine tetraacetic acid (EDTA), 10 mM Tris–HCl (pH8.5), 0.1% SDS, and 10 mg/ml DiThioTheitol (DTT)] under constant agitation at 42 °C. After that, samples were centrifuged (3000 rpm for 10 min) and supernatants were diluted with sterile water and treated with phenol–chloroform–isoamylalcohol. Finally, DNA was concentrated on a Centricon 30 column (Amicon, Racine, WI).

### *mtDNA amplification and sequencing*

Three riding pair primers (16034/16223, 16170/16340, 16277/16477) were designed to amplify the Hypervariable Segment 1 (HVS1) of the mtDNA control region:
L16034: GGGAAGCAGATTTGGGTACC; H16223: GGGTTGAT TGCTGTACTTGCT and L16170: AATCCACATCAAAACCCCCT; H16340: TGTGCTATGTACGGTAAATGGCT; L16277: ACCAACAA ACCTACCCACCC; H16477: CTACCCCCAAGTGTTATGGGC.

The primers allow the amplification of 189, 170, and 200 base pair (bp) of HVS1 mtDNA from nucleotide position 16034 to 16477. PCR amplification was performed in the ABI prism 7700 Sequence detection system (Applied Biosystem, Marly-le-Roi, France) using optimal conditions determined previously; PCR product was purified with Nucleospin Extract Kit (Macherey Nagel, Dueren, Germany) and then sequenced. The sequencing reaction was performed on the automatic sequencer ABI 3100 (Applied Biosystem, Life Technologies SAS, Marly-le-Roi, France) using the Big Dye Terminator v.3.1 (Applied Biosystem, Life Technologies SAS, Marly-le-Roi, France). The sequencing was done using the following program: one minute at 96 °C using 25 cycles: 10 s at 96 °C, 5 s at 50 °C, and 4 min at 60 °C.

### *Bioinformatic analysis*

HVS1 sequences were aligned and compared with rCRS sequences using 'Blast 2 sequence' software (Andrews et al. 1999; Tatusova & Madden 1999) and Seqscape software (V2.7) (Applied Biosystems, Life Technologies SAS, Marly-le-Roi, France). Sequences were sorted into haplogroups following Van Oven and Kayser (2009) and HaploGrep software (Kloss-Brandstätter et al. 2011).

Diversity parameters and the pairwise Fst were calculated using the software Arlequin ver. 3.5 (Berne, Switzerland) (Excoffier et al. 2005). The pairwise Fst can be used to describe the short-term genetic distance between populations with the application of a slight transformation to linearize the distance with population divergence time (Slatkin 1995). Kimura 2P distance option was selected.

The Fst matrix, was used with the statistical package for the social science (SPSS, version 13.0, Chicago, IL) to visualize data in multidimensional scaling (MDS).

## Results

### *Molecular analysis of AFA specimens*

Successful amplifications were observed mainly for fragments obtained with primer combinations: 16170/16340 (170 bp) and 16034/16223 (189 bp), whereas only one sequence was obtained with the primer 16277/16477 (200 bp). No amplification was obtained with the primer combination 16170/16470 (300 bp).mtDNA sequences of AFA individuals were aligned with rCRS, resulting haplotypes and haplogroups are described in Table 1.

The same HVS1 sequence was observed for each sample of AFA, extracted and analysed by independent experimenters in two independent laboratories. This observation highlighted the reproducibility of results.

Table 4. Diversity measures within mtDNA HVS1 in Taforalt and Afalou.

| Sample | Number of individuals | Number of different sequences | Number of variable sites | Number of transition | Number of transversion | Sequence diversity |
|---|---|---|---|---|---|---|
| Taforalt | 21 | 11 | 11 | 11 | 0 | 0.7810 ± 0.0943 |
| Afalou | 7 | 4 | 5 | 5 | 0 | 0.8095 ± 0.1298 |

The seven individuals of AFA showed five different haplotypes, classified into four haplogroups. Three specimens (AF2, AF13, and AF XXV) presented the same sequence of the reference: rCRS and could belong to H or U haplogroup. Two individuals (AF3 and AF7) were classified as haplogroup J and J1c3f. One individual (AF19) was classified as Haplogroup T2b. The last individual (AF22B) carrying the substitution 16126C could be classified as haplogroup JT or H14b1.

Interestingly among the seven samples tested, no Sub-Saharan haplogroups (L0–L7) were identified.

### Statistical and phylogenetic analysis

Diversity parameters (number of different sequences, number of variable sites, number of transition and transversion, haplotype diversity) of TAF and AFA samples are described in Table 4. The HVS1 mtDNA diversity observed in samples from TAF and AFA (0.7810 ± 0.0943 and 0.8095 ± 0.1298, respectively) was slightly lower than that observed in the majority of current Mediterranean populations (from 0.853 ± 0.045 to 1.000 ± 0.005; Kefi et al. 2015) and higher than that observed in Eastern Tuareg population (0.677 ± 0.046; Ottoni et al. 2009).

The matrix of Fst relating the 61 contemporary populations from North Africa, Europe, and Near East with the prehistoric individuals from TAF and AFA was reported in the supplementary material (S1). The matrix of significant Fst *p* values was reported in the supplementary material (S2). In order to facilitate the description of the results, Table 5 reports the comparison of TAF and AFA populations with the 61 actual Mediterranean populations.

The Fst values ranged from 0 to 0.588. The highest Fst was observed between Galician (GAL) and Chenini-Douiret population (CHO) (S1). The nearest population to TAF is Catalan (CAT) (Fst = 0.001) and the most distant is Bou Saâd (SAB) (Fst = 0.308). The highest genetic distance for AFA population is observed with Matmata (MAT) population (Fst = 0.254) (Table 5).

Based on Fst *p* values, we observed a statistically significant difference between AFA and Kesra (KES), Bou Omrane (OMB), MAT, Algerian Mozabite (MOZ), Saharawi (SAH), and Fezzan (Al Awaynat) (FAL) populations. Other populations are not significantly different from AFA samples (Table 5).

An MDS plot is constructed with the Fst genetic distances connecting the 61 current Mediterranean populations with the Iberomaurusian samples from TAF and AFA (supplementary material S1 and S3). Three populations (CHO, Bou Saâd (SAB), and Algerian MOZs) appeared to be outliers (supplementary materials S3) and are removed from the analysis (S4 and S5).

The new MDS plot (Figure 2) showed that TAF is close to Valencians (VAL), Minorcan (MIN), and Chuetas (island of Majorca) (CHU) in Spain, Berbers from Jerba in Tunisia (JEB), Sardinians (SSP and SAT) in Italy and Berbers from Northern Morocco (MBN) populations. Afalou samples are close to CHU, JEB, MIN, and SSP populations.

## Discussion

### DNA sequence authentication

Many arguments highlight the authenticity of the ancient DNA sequences of TAF and AFA populations performed in our study. First, skeleton from AFA and TAF was previously DNA quantified with chemiluminescent method. These samples harboured DNA ranged from 0.1 to 0.13 ng/µl, meaning DNA retrieved in bone up to 130 ng/g of bone (Stevanovitch et al. 2001). Moreover, Beraud-Colomb et al. (1995) had successfully amplified the *beta-globin* gene and the *amelogenin* gene from skeleton of TAF which confirms a good DNA preservation in these samples. Second, we followed recommendations for studies performed on ancient DNA. We took laboratory precaution to avoid contamination with current Human DNA (i.e. pre-PCR steps were carried out in a building where no molecular analysis had been performed previously; PCR and post-PCR laboratories were separated). Third, negative controls included mock extracts and PCR blanks failed to yield any amplification product. Fourth, successful amplifications of TAF and AFA individuals were observed only for fragments <193 bp. Fifth, all AFA and TAF mtDNA polymorphisms were different from those of operators. Sixth, DNA extracts obtained by each team (Faculty of Science, Luminy and Laboratory of Anthropology in the Faculty of Medicine) and analysed by different investigators, using their own batch of reagents yielded the same DNA sequence, which demonstrated that the results could be independently replicated. Finally, our results have a phylogenetic and phylogeographic sense: all HVS1 sequences of TAF and AFA are classified in mitochondrial haplogroups described in North African populations.

### On the origin of the Iberomaurusians

As they are hinges between the Palaeolithic and the Neolithic, the study of Iberomaurusian populations of TAF (Morocco, 23,000–10,800 YBP) and AFA (Algeria 15,000–11,000 YBP) provides important data in the understanding of the settlement of North Africa.

Our results showed that the mtDNA sequences of the seven specimens from AFA are classified exclusively into Eurasiatic haplogroups: H or U (three individuals), T2 (two individuals), JT (one individual), and J (one individual). Our findings are in agreement with our previous study performed on TAF population which reported a genetic structure

**Table 5.** Fst values and *p* values relating Taforalt and Afalou to 61 current Mediterranean populations.

| | | Taforalt (TAF) | | Afalou (AFA) | |
|---|---|---|---|---|---|
| Populations | Code | Fst | *p* Value | Fst | *p* Value |
| El Alia | ELA | 0.00699 | NS | 0 | NS |
| Qalaat El Andalous | QAL | 0.09374 | S | 0.06817 | NS |
| Zriba | ZRI | 0.02503 | NS | 0 | NS |
| Slouguia | SLO | 0.00831 | NS | 0 | NS |
| Testour | TES | 0.01517 | NS | 0 | NS |
| Capital Tunis | CTU | 0.04374 | S | 0.02486 | NS |
| Northern Tunisia | TUN | 0.02500 | NS | 0 | NS |
| Kesra | KES | 0.08522 | S | 0.07729 | S |
| Skira | SKI | 0.02446 | S | 0 | NS |
| Jerba Arabs | JEA | 0.05492 | S | 0.01101 | NS |
| Jerba Berbers | JEB | 0.00934 | NS | 0 | NS |
| Chenini-Douiret | CHO | 0.13056 | S | 0.05134 | NS |
| Sened | SEN | 0.06133 | S | 0.02375 | NS |
| Bou Omrane | OMB | 0.11617 | S | 0.15277 | S |
| Matmata | MAT | 0.00788 | NS | 0 | NS |
| Bou Saâd | SAB | 0.30850 | S | 0.25468 | S |
| Algerians | ALG | 0.08473 | S | 0.06507 | NS |
| Algerian Mozabites | MOZ | 0.12412 | S | 0.16028 | S |
| Algerian Mozabites | MOA | 0.06275 | S | 0.05179 | NS |
| Northern Moroccan (Berbers) | MBN | 0.01649 | NS | 0 | NS |
| Southern Moroccan (Berbers) | MBS | 0.03117 | S | 0.02146 | NS |
| Saharawi | SAH | 0.07010 | S | 0.08965 | S |
| Asni Berbers | ASB | 0.01995 | NS | 0 | NS |
| Bouhria Berbers | BOB | 0.01612 | NS | 0 | NS |
| Figuig Berbers | FIB | 0.07195 | S | 0.07056 | NS |
| Marrakech | MAR | 0.05926 | S | 0.05174 | NS |
| Upper Egypt | EGY | 0.07765 | S | 0.02779 | NS |
| Gurna | GUR | 0.12512 | S | 0.08265 | NS |
| Siwa Berbers | SIB | 0.04109 | NS | 0.00764 | NS |
| Alexandria | ALX | 0.03036 | NS | 0 | NS |
| Fezzan (Al Awaynat) | FAL | 0.05702 | S | 0.10570 | S |
| Fezzan (Tahala) | FTA | 0.10374 | S | 0.07803 | NS |
| Taforalt | TAF | 0.00000 | 0 | 0.06694 | NS |
| Afalou | AFA | 0.06694 | NS | 0.00000 | 0 |
| French | FRE | 0.01408 | NS | 0.00872 | NS |
| Andalusian | AND | 0.01308 | NS | 0.03919 | NS |
| Catalan | CAT | 0.00134 | NS | 0 | NS |
| South Italy | ITS | 0.00848 | NS | 0 | NS |
| Sardinian | SAR | 0.02391 | S | 0.02009 | NS |
| Sicilian | SIC | 0.00377 | NS | 0.01461 | NS |
| Greeks | GRE | 0.01757 | NS | 0 | NS |
| Centre Corsica | COR | 0.02280 | S | 0.04736 | NS |
| Southern Corsica | COB | 0.02383 | S | 0.03654 | NS |
| Central Sardinia | SAN | 0.01289 | NS | 0.03244 | NS |
| Northern Sardinia | SNS | 0.02033 | S | 0.04779 | NS |
| Sardinian (Sant' Antioco island) | SSA | 0.01065 | NS | 0.00113 | NS |
| Sardinian (Trexenta) | SAT | 0.01270 | NS | 0 | NS |
| Sardinian (San Pietro island) | SSP | 0.02201 | NS | 0 | NS |
| Tuscan | TUE | 0.00090 | NS | 0.02185 | NS |
| Northern Greeks | GNG | 0.01848 | NS | 0 | NS |
| Cypriots | GRC | 0.01879 | NS | 0 | NS |
| Galician | GAL | 0.00223 | NS | 0.00640 | NS |
| Basque | BAS | 0.02677 | S | 0.07093 | NS |
| Andalusia (Granada Province) | AGP | 0.01884 | NS | 0.03094 | NS |
| Majorcan | MAJ | 0.01178 | NS | 0 | NS |
| Minorcan | MIN | 0.04023 | S | 0 | NS |
| Valencian | VAL | 0.00787 | NS | 0 | NS |
| Ibizan | IBI | 0.05713 | S | 0 | NS |
| Chuetas | CHU | 0.03487 | S | 0 | NS |
| Turks | TUR | 0.01175 | NS | 0 | NS |
| Syrian | SYR | 0.02045 | S | 0 | NS |
| Palestinian/Israeli | PAL | 0.02043 | S | 0 | NS |
| Druze | DRU | 0.10172 | S | 0.06741 | NS |

S: significant; NS: not significant; significance level=.05.

composed mainly of Eurasiatic haplogroups (Kefi et al. 2005). Indeed, 19 among 21 individuals of TAF, are classified as Eurasiatic haplogroups (H, U, JT, V). The two remaining individuals belong to the North African haplogroup U6.

The absence of haplotype belonging to Sub-Saharan haplogroups (L0–L7) would suggest that our sample of Iberomaurusians is not originating from Sub-Saharan region. These results confirm dental, craniofacial, post-cranial comparative studies, and industry investigations which found divergence between Iberomarusian skeletons and their contemporaneous Nubians (Camps 1974; Ferembach 1985; Bermudez de Castro 1991; Irish 2000).

The distribution of the Sub-Saharan component in the current North African populations ranged from 3.2% in Moroccan from Souss region to 43% in Mauritanian (Brakez et al. 2001; Plaza et al. 2003; Gonzalez et al. 2006; Kefi et al. 2015). The absence of the Sub-Saharan component in our Iberomaurusian samples suggests a recent gene flow from South to North Africa (at least after 10,000 YBP). This agrees with an analysis of STR/Alu combination polymorphisms that suggests that the Sub-Saharan component of current North Africans could be traced back to the first stage of Neolithic (around 9000 YBP) characterized by an ethnic contribution from present-day Sudan (El Moncer et al. 2010).

Our phylogenetic analysis showed that Iberomaurusian individuals from TAF and AFA (coastal archaeological sites in Northern Morocco and in Northern Algeria respectively) are genetically close to Berbers from the North of Morocco, Berbers from the Jerba Island in Tunisia and close to some South Western European populations: Valencia and the Balearic Islands from Spain and Sardinia from Italy (Figure 3). This finding highlights the existence of a broad Mediterranean mitochondrial gene pool including population from North Africa and South Western Europe. Around 24,000 years BP, the level of the Mediterranean was less than 110 m compared to the current level (Ferembach 1985) that would have facilitated population movements between these regions.

## Genetic continuity

All haplogroups observed in individuals from TAF and AFA are found in contemporary North African populations (Plaza et al. 2003; Coudray et al. 2009; Ottoni et al. 2009; Ennafaa et al. 2011; Kefi et al. 2015). Moreover among the current North African populations studied to date, the genetic structure of the Berber population of Northern Morocco presents similarities with the population of TAF: These Berbers have the lowest rate of sub-Saharan haplogroups (3.2%) as TAF population. Also, all haplogroups observed in TAF are found in this current population, even the rare haplogroup J/T. This J/T haplogroup, represented at 1.6% in the Northern Moroccan Berber population, is only represented in Sicilian (1.8%) and in other Italian populations (1.6%) (Pinto et al. 1996; Rando et al. 1998; Richards et al. 2000b; Cali et al. 2001; Plaza et al. 2003).

In addition, among Mediterranean populations, only one U6 sequence, observed in Moroccan individual (16172C–16174T–16304C) (Rando et al 1998), could be related to an haplotype observed in the population of TAF (16172C–16174T).

Our molecular and phylogenetic results suggest a genetic continuity in North Africa and the existence of genetic flows

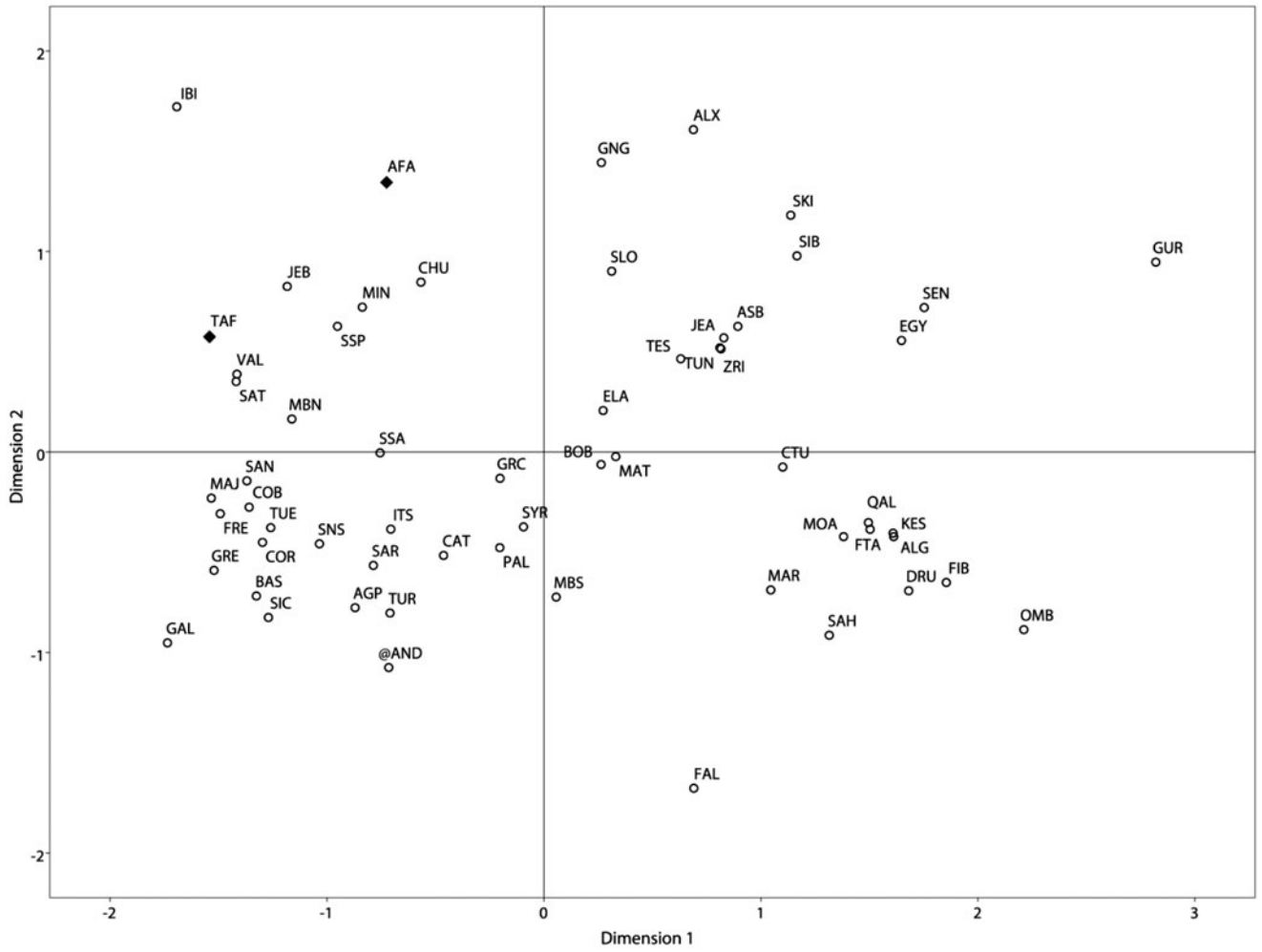


Figure 2. Position of Taforalt and Afalou populations within Mediterranean populations (without outlier populations: MOZ, SAB, CHO) using MDS plot method.


Figure 3. Geographical representation of current populations that are genetically close to Taforalt.

between both sides of the Mediterranean. Our results confirm previous dental investigation and anthropological studies suggesting long term population continuity in the Maghreb (Ferembach 1986; Irish 2000).

### *The chronology of the Eurasiatic gene flows in North Africa*

Previous studies performed on current populations showed that the majority of the Eurasian haplogroups such as T, H, J are originated in Near East during the Palaeolithic. JT arose ~58,000 years ago. J and T diverged ~40,000 years and ~30,000 years ago, respectively and started to spread from the Near East to Europe immediately after the peak of the last glaciations, ~19,000 years ago (Richards et al. 2000a; Pala et al. 2012). H Sub-haplogroups (H1, H3, H5), V and U5b are the signatures of postglacial expansion from the Iberian Peninsula into the European continent and North Africa (Ottoni et al. 2010).

According to our results, the presence of Eurasian haplogroups (JT, J, T, H, R0a1, U) in AFA and in TAF individuals suggests that these lineages were present in North Africa at least 21,000 YBP confirming the estimated coalescence time for these haplogroups (Brandstätter et al. 2008; Ennafaa et al 2009; Ottoni et al., 2010; Pala et al. 2012; Zheng et al. 2012).

## Conclusions

The analysis of mtDNA variability in prehistoric populations from North Africa has been a powerful tool to understand the origin of North African settlement. The study of the Iberomaurusian samples of AFA and TAF excludes the Sub-Saharan origin of these individuals. In addition, this study highlights the existence of broad Mediterranean mtDNA gene pool between the Southern and the Northern coast of the Mediterranean since the Epiplaeolithic period.

In perspective, we will extend this molecular study of the population of AFA on other new specimens, since the excavation of this prehistoric site is still under progress. The study of ancient DNA from Neolithic populations such as the Capsian population will be very helpful to precise the chronology of Sub-Saharan gene flow in North Africa.

The presence of J/T haplotypes at 21,000 YBP could be further investigated in an another ancient population to precise the link between North Africa and Near East.

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## Disclosure statement

The authors report no declarations of interest.

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