Characterisation of Mitochondrial DNA and Y-STR Variation in the Libyan Population

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A Thesis Submitted to the School of Applied Sciences at the University of Huddersfield, in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Population Genetics

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Abstract

Despite the distinct strategic geographical location of Libya linking North Africa with the Middle East and Europe with sub-Saharan Africa and rich human settlement history, the genetic diversity in Libya has not been well studied compared to other North African populations.

To shed light on the genetic composition of the modern populations in Libya and investigate human migration patterns and arrival time of different ethnic and geographic groups, samples were collected and analysed for 3 ethnic groups including Berber, Arab and African groups in Libya. 375 hypervariable sequence mitochondrial DNA (mtDNA) fragments were analysed by Sanger sequencing, 199 samples were selected for whole mitogenome next-generation sequencing and 219 samples had 23 Y chromosomal STR loci variations analysed.

Data analysis showed that both female and male lineages in Libya correlated with their geographical region in North Africa. However, the analysis showed that both lineages experienced a different pattern of gene flow that led to the absence of correlation between their ancestral origin where mitochondrial DNA showed a dominance of Eurasian lineages including N1, R0, HV, V, H, J, T, U, K, and X and moderate influence of sub-Saharan lineages including L0, L1, L2, L3, and L4. The paternal genome-wide diversity was mainly constituted by dual components; indigenous North African lineage haplogroup E-M81 and the Arabic components J(xJ1a, J2)-M304. The analysis also showed that the distributions of some maternal lineages statistically correlated with ethnicity such as haplogroup U6 for Libyan Berber, haplogroup L for African Libyans and haplogroups R0a for Arab group. Furthermore, founder analysis of complete mtDNA genome show that all the Berber populations have local roots involved in the first settlement of the current population in Libya during Iberomaurusian period ~22,000 years ago including haplogroups U6 and M1 and have received multiple maternal lineages from West Europe during the last glacial warming, Levant components during the Holocene and in Neolithic associated with the spread of agriculture. Neutrality tests confirmed the evidence of a recent population expansion in Libya that is probably

attributed to the major Arab arrival in 7th and 10th centuries. Age estimation and founder analysis of mtDNA also revealed that the majority of current sub-Saharan lineages found in Libya were probably recently introduced associated with the trans-Saharan slave trade that was started in the seventh century. In addition, there were a few lineages that were most likely introduced in Libya with the spread of Iberomaurusian expansion culture from the Levant (haplogroup L2) and during the early Holocene after a humid period (haplogroup L1).

With regards to the analysis of the Libyan Berber populations maternal lineages, the Zuwara population experienced high levels of genetic drift resulting in a small number of haplotypes elevated to high frequencies as demonstrated by haplogroups J1c. In contrast, the Libyan Arabs being relative newcomers to Libya had much higher levels of diversity among them.

Overall, the newly presented data in this study refines the knowledge about Libyan population history and serves as a basis for comparison with other populations and contributes to the establishment of databases suitable for forensic casework where mtDNA and Y-STR information might be helpful.

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List of Abbreviations

AMHS	Anatomically modern Homo sapiens
AMOVA	Analysis of molecular variance
ATP	Adenosine triphosphate
BC	Before Christ
BEAST	Bayesian evolutionary analysis by sampling Trees
Bp	Base pairs
Bp	Before the present
BSP	Bayesian skyline plot
CE	Common era
CRS	Cambridge reference sequence
D-loop	Displacement loop
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside triphosphates
HKY	Hasegawa/Kishino/Yano
HTUS	Hypothetical taxonomic units
HVR1	Hypervariable region 1
HVR2	Hypervariable region 2
HVR3	Hypervariable region 3
Kya	Thousand years ago
LGM	Last glacial maximum
LHON	Leber's hereditary optic neuropathy
MCA	Multiple component analysis
MCMC	Markov Chain Monte Carlo

MDA	Multidimensional scaling
ML	Maximum likelihood
MP	Maximum parsimony
MRCA	The most recent common ancestor
MSY	Male-specific region of the Y chromosome
MtDNA	Mitochondrial deoxyribonucleic acid
NGM	Next generation multiplex
NJ	Neighbour-joining
Np	Nucleotide position
NRY	Non-recombining (region) of Y-chromosome
NUMTS	Nuclear inserts of mitochondrial DNA
OTUS	Operational taxa unite
PAR	Pseudoautosomal region
PCA	Principle component analysis
PCR	Polymerase chain reaction
Rcrs	The revised Cambridge reference sequence
RFLP	Restriction fragment length polymorphism
SNPs	Single nucleotide polymorphisms
STR	Short tandem repeat
TMRCA	Time of most recent common ancestor
YCC	Y-chromosome consortium
Y-DNA	Y chromosome deoxyribonucleic acid YHRD
YHRD	Y-Chromosome STR haplotype reference database

Chapter 1

Introduction

1. Introduction

1.1. General background

There is great interest in the origins of modern humans and where our ancestors came from and timing of these events. At the beginning of research, human population studies depended on the archaeological, fossil and linguistics. These studies were supported by anthropological and historical information to elucidate the origin and how humans spread across the world (Jobling et al., 2013a). In the past three decades, methods that utilise genetic information have also helped to predict human origin and migration (Tishkoff et al., 2007). This genetic information contained in non-recombining mitochondrial DNA (mtDNA) and the Y-chromosome make them ideal markers for human evolutionary studies. This feature also allows scientists to investigate evolutionary events such as movements, founder events, population expansions, marriage behaviours, and mode of language transmission (Wilson et al., 2001). Most of the anthropological and genetic studies suggested that modern humans originated in Africa (Lahr and Foley, 1998, Jobling et al., 2013a). However, they differed in the estimation of the most recent common age of the maternal ancestor (mitochondrial Eve), while it is estimated at nearly 200 kya using the Mishmar coding-region rate for mitochondrial DNA (Mishmar et al., 2003), it is only 160 kya using Kivisild‘s synonymous rate (Kivisild et al., 2006). More recently, the estimate for the female most recent common ancestor (MRCA) is about 190 kya using complete mtDNA genome data (Soares et al., 2009).

The age of the paternal ancestor (Adam) of modern humans using more than 10,000 male-specific Y-chromosome single-nucleotide polymorphisms (MSY-SNPs) is estimated between 180 kya and 200 kya years (Cann, 2013). A different study conducted by Poznik, using 9.99 million loci on the Y chromosome this was estimated the male MRCA to be between 120 kya to 156 kya (Poznik et al., 2013). These estimates roughly agreed with dates obtained from the analysis of the mitochondrial genome for MRCA (Cann, 2013).

1.2. Mitochondrial DNA

Mitochondria are semi-autonomously functioning organelles possess by eukaryotic organisms, present within the cytoplasm. Human mtDNA is a circular supercoiled double-stranded haploid genome normally 16,569 bp in length, which consists of the pyrimidine-rich (light) strand and purine-rich (heavy) strand, passed down through the maternal lineage (Jobling et al., 2013a). As mitochondria have their genetic code; this strengthens the hypothesis that mitochondria mtDNA initially originated from endosymbiotic bacteria then taken up into eukaryotic cells 1.5 billion years ago (Embley and Martin, 2006).

Excepting, red blood cells there are hundreds of mitochondria in each cell, and 2-10 copies of mitochondrial DNA occurs within each mitochondrion organelle (Jobling et al., 2013a). In contrast to nuclear DNA, about 93% of the mitochondrial genome is a coding region that comprised of 37 genes encoded for 13 proteins essential for the electron respiratory chain of the mitochondria including ATPase subunit, cytochrome c oxidase subunits I, II and III, and cytochrome b (Anderson et al., 1981). It also codes for 22 tRNAs and two rRNAs (12S and 16S) required for the polypeptides synthesis (Penta et al., 2001). However, the majority of proteins that are needed for the division and function of the mitochondria are encoded by the nuclear genome (Lodish, 2008). An illustration of the mitochondrial genome is shown in Figure 1.

The numerical designation for mtDNA starts at the heavy strand origin of replication and continues around the circle for 16,569 bp (Andrews et al., 1999, Holt and Reyes, 2012). The 1120 bp non-coding region within the mitochondria genome termed the D-loop or control region is located between the mitochondrial tRNA-Pro and tRNA-Phe genes (Figure 1). It contains one primary origin of replication and several secondary origins of replication (Jobling et al., 2013a).

The control region contains hypervariable regions that are evolving faster than the coding region of the mitochondrial genome. The hypervariable region is split into three parts. Hypervariable region I (HVI) which extend from position 16,024 to 16,365, hypervariable region II (HVII) ranges from 73-340, and hypervariable region III is located from position 438 to 574. These boundaries vary

slightly between laboratories. These HV1 and HVII regions are also known as hotspots for nucleotide substitutions, (Parson et al., 1998, Wallace, 1999).

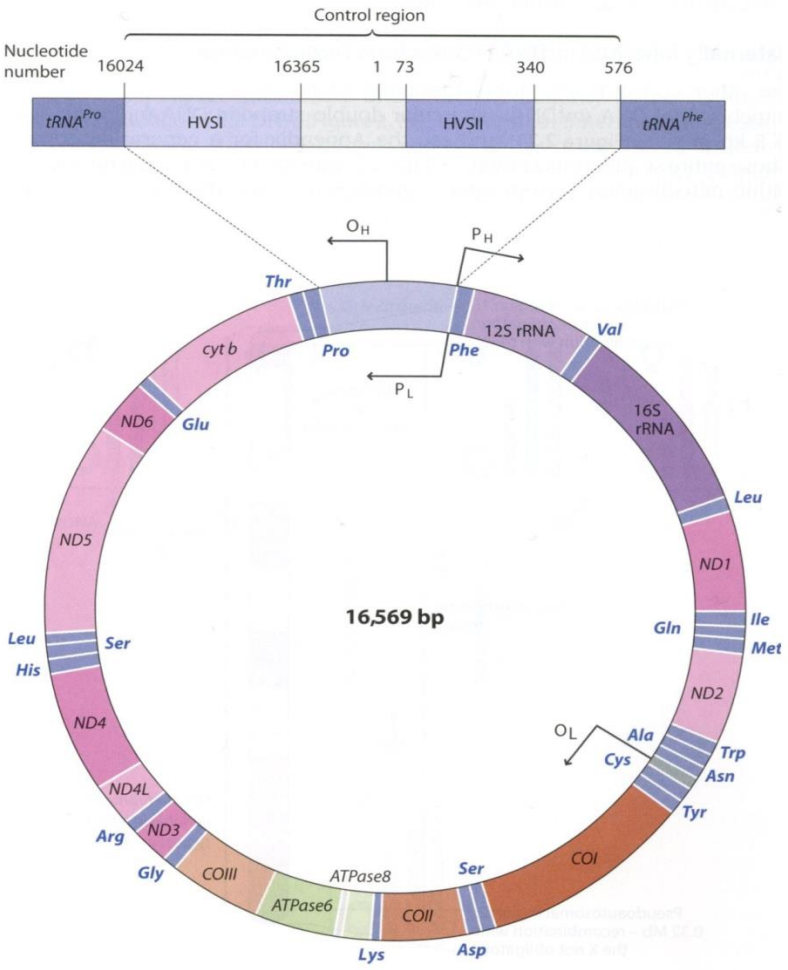

Figure 1. Human mitochondrial genome

(Jobling et al., 2013a)

1.2.1. mtDNA division and replication

During the lifetime of a cell, mitochondria divide several times according to the cell's requirements. This division allows for the complete correction of the number of mitochondria during the cell division (Embley and Martin, 2006). Both heavy and light strands of mtDNA contain two different origins of replications that are used by mitochondria (Penta et al., 2001). The mitochondrial mechanism of DNA transcription and replication differs from nucleus DNA, where mtDNA

replication is virtually stopped during the first step of cell division when the cells are kept in a metaphase stage until fertilisation and cleavage occur (John et al., 2010). The replication then starts after the implantation process, and cell differentiation and proliferation begins in the blastocyst, offering an environment for the generation of mtDNA mutations. Transcription and replication regulation of mitochondria occur by multiple promoters and protein-binding domains that contained in the mitochondrial control region (Pakendorf and Stoneking, 2005).

1.2.2. mtDNA transmission and inheritance

Mitochondrial DNA is strictly transmitted through a maternal line of descent (Chinnery, 2006). Sperm mitochondria can reach the oocyte during fertilisation period, but they then quickly degraded prior to implantation. Some doubts have been raised about the strict maternal inheritance of mtDNA (Pakendorf and Stoneking, 2005, Maca-Meyer et al., 2001). As a single case of paternal inheritance of mtDNA has been discovered (Kvist et al., 2003, Kraytsberg et al., 2004). However, it has been suggested that this case might be due to a breakdown of the normal system. Thus, so far, paternal inheritance is considered as a rare event, and maternal inheritance of mtDNA in humans are the standard mode of inheritance (Manfredi et al., 1997, Cummins et al., 1998, McVean et al., 2002).

1.2.3. mtDNA recombination

mtDNA has several unique features, which has made it the preferred molecule for human evolutionary studies, these characteristics include maternal inheritance, lack of recombination and high rate of mutation (Jobling et al., 2013a). Recombination that occurs in the autosomal diploid nuclear genome is defined as the exchange of nucleotide sequences during meiosis, and is a process that influences biological evolution and explains the continuous increasing of genetic diversity among populations. In contrast, the haploid mitochondrial DNA does not recombine, is transmitted stably from generation to generation; but this can be interrupted by mutations (Jobling et al., 2013a).

1.2.4. Heteroplasmy of the mitochondrial genome

When there is a mutation in a mtDNA molecule, that molecule will replicate in parallel with the non-mutated mtDNA, resulting in a mixture of normal and mutant mitochondrial molecules. Cells, which contain a mixture of mutant and maternal mtDNA, are called heteroplasmic (Ruokonen and Kvist, 2002).

Human mtDNA is usually present in a homoplasmic state within an organism; however when heteroplasmy occurs, and heteroplasmic cells divide, the proportion of normal (parental) to mutant molecules passed to progeny cells can vary according to replicative segregation; offspring cells may remain heteroplasmic or in the extreme conditions, may become homoplasmic, only containing mutated or the normal mtDNA (Coble et al., 2004). mtDNA in tissues with a slower turnover tend to gather more heteroplasmic DNA than speedily replicating lineages like white blood cells. Homoplasmy of the defective mutant mtDNA can be lethal if a mutation in mtDNA cells adversely affects mitochondrial function. Usually, mitochondrial disease phenotypes will not be expressed until the threshold of mutant mtDNA extends to at least 60%. This threshold can vary however according to the type of tissue as the energy requirement is different within the cells (Jobling et al., 2013a).

During each maternal mitochondrial transmission, there is a fast intergenerational change within a mitochondrial gene as well as levels of heteroplasmy. This rapid change is thought to be as a result of random genetic drift. However, the nature of the genetic drift during oogenesis is not completely known (Jobling et al., 2013b).

Heteroplasmy occurs when the cell contains two different types of mitochondria or when two different types of mitochondria are found in different cells with the same organism. (Budowle et al., 2002, Stewart et al., 2001, Wilson et al., 2002).

Heteroplasmy can be further divided into a length heteroplasmy and sequence heteroplasmy. In the sequence heteroplasmy, there is a substitution of at least a one nucleotide base in the mtDNA genome (Paneto et al., 2007). In length heteroplasmy, a C-stretch region is the most frequent form,

where a T to C transition at position 16,189, leads to a stretch of 10 to 13 cytosines on the light strand of mtDNA. This phenomenon can complicate the interpretation of mtDNA analysis (Sullivan et al., 1996, Budowle et al., 2002, Tully et al., 2004).

1.2.5. Nuclear mitochondrial DNA segment (NUMTS)

As part of an ongoing evolutionary process genes are continuously transferred from the mitochondrial genome to the nuclear genome; also, some of the genes have possibly been erased during evolution due to loss of purpose in the symbiotic condition or redundancy with the nuclear genome (Hazkani-Covo et al., 2010). Integration of nuclear copies of mitochondrial DNA (numts) usually is considered as a neutral polymorphism; however, rarely it can be associated with human diseases. Numts have been present in the slowly evolving nuclear genome for possibly millions of years; therefore, they could be used as outgroups of the human mitochondrial tree; however, numts can be amplified results in a heterogenous mixture of nuclear and mitochondrial amplicons
might cause analytical problems and provide erroneous sequences of mtDNA (Hazkani-Covo et al., 2010), leading to wrong interpretations of population genetics, phylogeography and forensic genetics. The numts however only become a problem in the case of ancient DNA; since in the good quality mtDNA, the number of copies of mtDNA should greatly outnumber the copies of nuclear genome (Bensasson et al., 2001, Goios et al., 2008).

1.2.6. Mitochondrial DNA Cambridge reference sequence (CRS)

In 1981, the first complete reference sequence of the human mtDNA was obtained from two individuals. This sequence is known as the Cambridge reference sequence (CRS) (Anderson et al., 1981). Since then eleven sequencing errors were corrected (Andrews et al.,1999), and this revised version, which is used to compare with new mtDNA sequences, is known as Revised Cambridge Reference Sequence (RCRS) (Van Oven and Kayser, 2009).

1.2.7. Mutation in mitochondrial DNA

The inability, to repair damages in a DNA sequence, whether due to oxidation or other processes, can cause mutations. In mitochondria, a mutation can arise during replication; however, heritable mutations only occur in oocyte mitochondria. Several factors are believed to contribute to the relatively high rate of mutation in mtDNA are exposure to oxidative stress from the reactive oxygen species formed during oxidative phosphorylation (Fernández-Silva et al., 2003) and the lack of a proper DNA repair system and the fact that DNA in mitochondrial is less protected by than the nuclear DNA (Bohr et al., 2002, Turrens, 2003). Mutation in a mtDNA molecule in which one nucleotide is substituted by another is classified into two types:

Transition: one pyrimidine base (C or T) substituted for the other pyrimidine or purine base, substituted for the other purine (A or G).

Transversion: purine substituted for a pyrimidine or vice versa (Sobrino et al., 2005, Frazer et al., 2007, Consortium, 2010).

The other types of DNA mutations include insertions or deletions which are very common in the human genome, occurring on average once every 200-300 bp within the human population (Kruglyak and Nickerson, 2001, Gibbs et al., 2003).

1.2.8. Mitochondrial mutation rate

It is estimated that mtDNA has a ten times faster mutation rate than the nuclear DNA(Lightowlers et al., 1997). The mtDNA control region has ten times more variable sites than the mtDNA coding region. While the phylogenetic estimates for the HVS-I control region are 1.80×10^{-7} transitions per nucleotide per year, the coding region rate is 1.26×10^{-8} base substitutions per nucleotide per year (Mishmar et al., 2003).

This ratio is important to phylogenetic reconstructions and calculating genetic distances between mitochondrial sequences (Yang and Yoder, 1999).

However, the pedigree estimates are ten times faster rate than phylogenetic rates (Howell et al., 2003). The disparity cannot be attributed to one factor with many factors acting together that may cause this difference such as mutation, genetic drift, the lack of the ability of phylogenetic methods to capture the high levels of control-region homoplasmy and selection (Howell et al., 2003).

Natural selection and purifying selection, in particular, is probably an important factor determining mutation rates at different time depths (Penny, 2005, Henn et al., 2009). The high level of sequence variation within the control region has made it a target of choice for studies of human evolution and further has led to its use as a tool for human identification of victims of mass disasters and war crimes in forensic applications.

1.3. Mitochondrial DNA haplogroups

The strict pattern of maternal inheritance of mtDNA leads to a natural clustering of sequence haplotypes sharing common basal mutations into monophyletic groups called haplogroups. Individuals within the same haplogroup have a specific sequence because of sharing a closely related maternal lineage due to them having a common ancestor (Budowle et al. 2003). So, mitochondrial haplogroups are distinguished between groups of mtDNA mutations, both in non-coding and coding regions, comparing to the rCRS. These haplogroups can be traced back to common matrilineal or most recent common ancestor (MRCA) (Macaulay and Richards, 2008b, Behar et al., 2008). Mathematical formulas can be used to estimate the time and age of the sequence and transformed it into a tree known as a phylogenetic tree (Soares et al., 2009). Due to an accumulation of mutations in maternally lineages, all mtDNA haplogroups can be represented in one phylogenetic tree. Presently, the most comprehensive and regularly updated overview of the human mtDNA tree can be found in Phylotree (http:// www.phylotree.org) (Van Oven and Kayser, 2009, Kayser, 2007).

1.3.1. Nomenclature and distribution of the mitochondrial DNA haplogroups

The present nomenclature of mitochondrial DNA haplotypes was firstly introduced by naming of the main Native American haplogroups A, B, C and D by Torroni (Torroni et al., 1996) (Figure 2). Later, new haplogroups were added, and soon all the alphabet letters were utilised. All non-African mitochondrial lineages derive from M and N haplogroups that originate from the root of haplogroup L3 that has also given rise to some African population sub-clades (Underhill and Kivisild, 2007). The vast majority of the Western Eurasian mitochondrial lineages including Mediterranean Africa, contain haplogroup J, K, T, H, I, U, V, X and W (Torroni et al., 1996), while haplogroups M, B, F and A were found in the Mongoloid populations. Although the diversity in Africa is high, the African lineages were given a single letter L (Chen et al., 2000) (Figure 2). Macro-haplogroups that include a group of clades are named with uppercase like (D, L. M), and sub-haplogroups are defined with numbers and lowercase letters like (L1, J1c, L2e2). Some haplogroups are defined by merging two haplogroups names, such as HV (Richards et al., 2000b) (Figure 2).

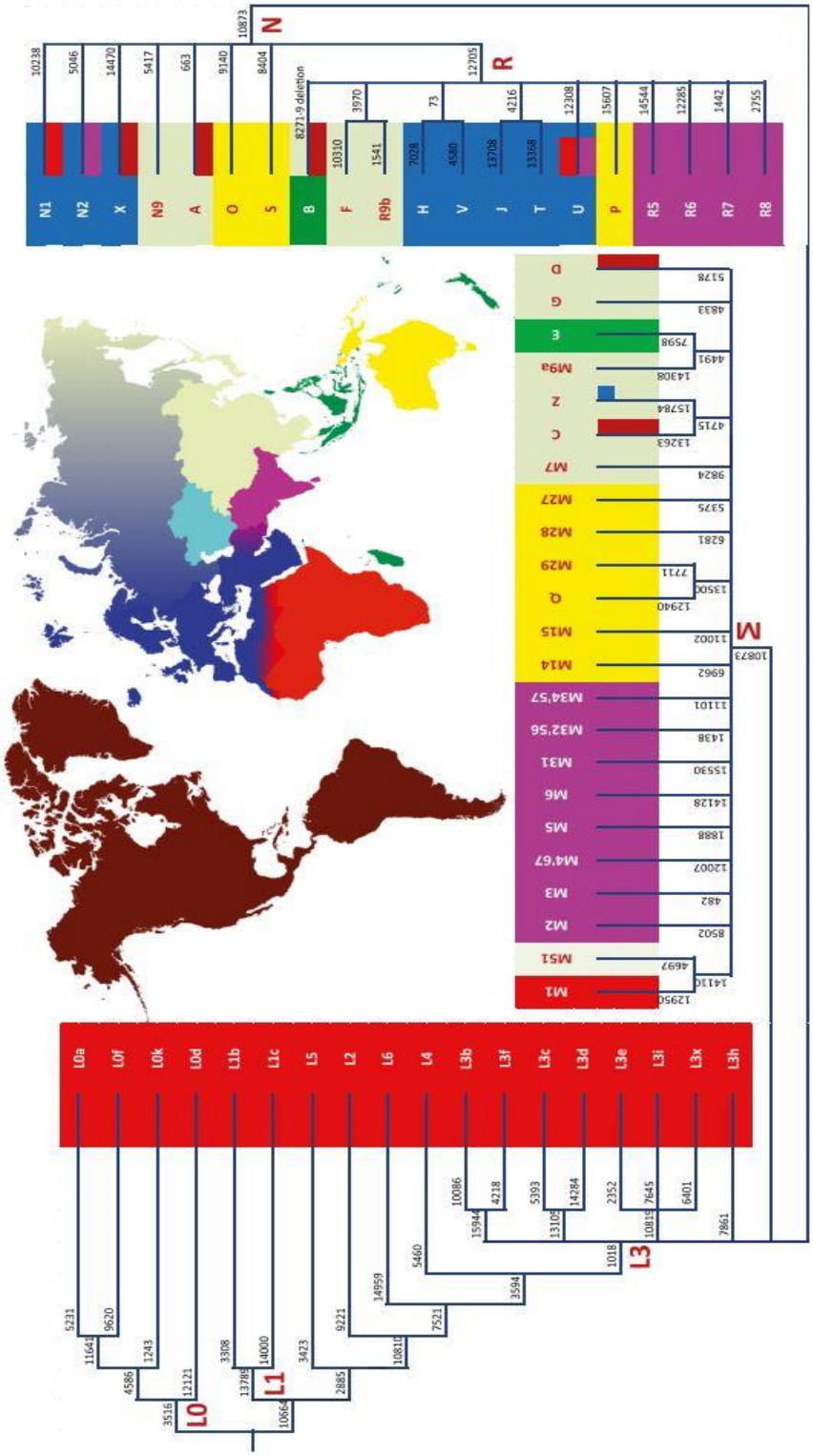

Figure 2. mtDNA haplogroup tree and distribution map.

Taken from (Kivisild, 2015)

Previous studies have found that the majority of human mitochondrial DNA variations are concentrated sequentially in the maternal lineages from sets of mitochondrial DNA founders, during

and after the human colonisation process all over the world. Scrutiny of mtDNA sequence information is crucial to distinguish variation from either sequencing artefacts or phylogenetically informative sites. The leading causes of 'phantom' mutations are contamination, biochemical problems, transcription error and sample mixup. A phantom mutation may lead to false associations and wrong conclusions. As a result, guidelines concerning mtDNA genotyping have introduced (Bandelt et al., 1995, Kloss-Brandstätter et al., 2011, Reid, 2013). The guidelines include double evaluation of mtDNA sequence. The other step is the detection of phantom mutations by the construction of quasi-median networks. The last step is checking for phylogenetic consistency by confirming the haplogroup classification (Bandelt et al., 2009, Kloss-Brandstätter et al., 2011).

1.4. The mitochondrial molecular clock

It is estimated that the mtDNA control region has one transition per nucleotide per 20,180 years for the HVS-I control region (Forster et al., 1996) while it is estimated there are 1.26×10^{-8} base substitutions per nucleotide per year in the coding region (Mishmar et al., 2003). However, the most commonly used mutation rate for the analysis of mtDNA HVS-1 has been 1.80×10^{-7} transitions per nucleotide per year(Forster et al., 1996).

Although the mutation rate was calibrated using the variation accumulated in the Eskimo and Na-Dene, the work with pedigrees has challenged the rate as being too slow (Parson et al., 1998, Howell et al., 2003). Since these mutations occur after a period of time, the mutation rates are like an evolutionary clock for the human mitochondrial DNA with a homogenous distribution of the mutation rate along the timescale of mtDNA phylogeny. In the coding region, the percentage of synonymous mutations are high in ancient tree branches comparing with the young branches and vice versa the young branches in the tree contain a higher percentage of nonsynonymous mutations than the ancient branches. The possible cause of this trend is the effect of purifying selection which is acting gradually over time on deleterious mutations (Elson et al., 2004, Kivisild et al., 2006, Pereira et al., 2006, Ruiz- Pesini and Wallace, 2006, Soares et al., 2009).

Many estimations of rates have been made for the synonymous substitutions. While that Kivisild (Kivisild et al., 2006) found a mutation average of one transition every 6884 years for synonymous substitutions, Soares et al. (2009) suggested a slower substitutions rate for synonymous mutations where one mutation occurs every 7884 years. Also, when Soares et al. (2009) built a phylogenetic tree using 2000 complete whole human genomes they estimated one mutation every 3624 years by correcting the rate mathematically for different time scales and the effect of purifying selection.
This mutation rate is interspecific and corresponds to the human-chimpanzee split. It is then further corrected mathematically for different time-scales and the effect of purifying selection. (Soares et al., 2009).

1.5. Founder ages and founder analysis

The principal aim of phylogeography is to identify the possible founder source of a particular population and to identify their time of arrival (Richards et al., 2000a)**.** A complete sequence is needed to obtain a full genealogical resolution; however, the control region still has enough information for building phylogenetic trees (Soares et al., 2009). It can link the sequences of the population under investigation to the common ancestors and calculate the founder age to evaluate the diversity among the general population. The founder age is calculated by identification of a founder sequence and then estimating the diversity of this particular population that occur over time using a phylogenetic tree or network (Richards et al., 2000a). The failure to understand the fundamental principles behind the calculation of a founder age may lead to estimates of the age of a clade in a region that can predate the age of colonisation of that region (Richards et al., 2000a).

1.6. Forensic use of mitochondria

Nowadays mtDNA is widely used in forensic cases, especially in a situation where nuclear DNA is degraded or in low quantities or poor quality. The high copy number of mt DNA increases the possibility for it to be recovered in a good amount from even a small sample (Butler, 2009). Furthermore, the circular nature of the mitochondrial genome provides a protective mechanism

against the breakdown by exonucleases and hence increases its survival potential (Butler 2005b). Moreover, mitochondrial DNA is inherited down the maternal lineage, and mtDNA passes unchanged from maternal generation to generation except in case of spontaneous mutations. As a result maternal relatives can help in solving mass disaster investigations and missing person cases even in the absence of the direct mother (Primorac and Schanfield 2000).

1.6.1. Steps involved in obtaining mtDNA results for forensics.

As mitochondrial DNA is present in a high copy number per cell, it is more susceptible to contamination than nuclear DNA. Therefore, it is important that extraction and analysis are performed in a very clean laboratory. Also, it is preferable to process samples before the reference samples to avoid any potential contamination (Goodwin et al., 1999). Analysis of mitochondrial DNA is usually carried out using the Sanger sequencing technique in both directions (forward and reverse). This ensures the sequence of all parts of the target segment and both complementary strands aligned together and compared for quality control purposes. mtDNA variations are reported as differences to the CRS. If the sequence sample under test containing a T nucleotide at position 16,126, which contains a C in the CRS this would be reported as 16126T. If the remaining of sequence does not contain other nucleotide variants, then it is suggested that the rest of the sequence is identical to the CRS (Budowle et al., 1999a, Goodwin et al., 1999).

1.6.2. Interpretation of mtDNA results in forensic casework.

Human mitochondrial DNA (mtDNA), is becoming routinely analysed in forensic genetics for typing small and degraded remains (Butler, 2009, Goodwin et al., 1999, Pereira et al., 2009).

After completion of mtDNA analysis results for the question (Q) and a reference sample (R) are compared in Figure 3 for both segments HV1 and HV2. All nucleotides (positions HV1 16,024 – 16,365 and HV2 73 – 340) are then evaluated between compared samples. Finally, mtDNA sequence results can be classified into three results based on the Q-K comparison and according to

the scientific working group on DNA analysis methods (SWGDAM) guideline recommendations for mtDNA interpretation into exclusion, inconclusive, or failure to exclude (Butler, 2009).

	16024	16120	16310	16320	16330	163140
rCRS	ACCGGTATGT	ATTTCGTACA	TTACTGTCAG	CCACTATGAA	TATTGTACGG	TACCATAGAT
Q	ACCGGTATGT	AT**C**TCGTACA	TTACTGTCAG	CCACTATGAA	TATTGTAC**A**G	TACCATAGAT
K	ACCGGTATGT	AT**C**TCGTACA	TTACTGTCAG	CCACTATGAA	TATTGTAC**A**G	TACCATGAAT

(b) Reporting Format with Differences from rCRS

Sample Q	Sample K
16120C	16120C
16330A	16330A

Figure 3. mtDNA Sequences Aligned with rCRS (positions 16071–16140)

Taken from (Butler, 2005)

Exclusion: When there are more one nucleotide variants between the reference and questioned samples, the evidence sample can be excluded because it originated from another maternal lineage.

Inconclusive: If there is only one nucleotide difference between the questioned and reference samples, the result, in this case, will be inconclusive. The single nucleotide difference is classified regarding an inconclusive result because of mutations observed between mother and children. For instance, if a maternal relative is used as a reference, the chance of a single base difference might exist between two samples that are, in fact, maternally related. In such these cases, more reference samples are run in an attempt to ensure the correct interpretation (Butler, 2009).

Cannot exclude: If both evidence and reference samples under comparison have the same sequence at each position or the same length variation, in the HVR, C-stretch, the sequences cannot be excluded as descending from the same maternal line (Budowle et al., 1999a, Goodwin et al., 1999).

A statistical calculation of the significance of a match is always needed when cannot exclude' is the interpretation.

1.7. Mitochondrial genetic diseases overview

While some genetic variants have little or no apparent impacts, others have serious consequences on an individual's phenotype and disease susceptibility; also, the variations can determine an individual response to drug treatment (Guttmacher et al., 2003, Evans and Relling, 2004). Genetic variants can directly lead to disease by altering protein function or changing gene dosage; however, these are usually rare diseases, and most common diseases are, multifactorial, caused by a combination of participating of genetic and environmental factors (Mathew, 2001 and Dean, 2003).

Mitochondrial disease refers to a disorder in which the aetiology is a defective mitochondrial respiratory chain (MRC) resulting in a defect in oxidative phosphorylation. Although mtDNA is maternally inherited, the functioning of mitochondria is dependent on many nuclear genomes encoded factors. As a result, mitochondrial disease can be inherited in maternal autosomal dominant, autosomal recessive, or X-chromosomal fashion (DiMauro and Davidzon, 2005).

The sequence variation in human mitochondrial DNA is mainly considered to be neutral. However, some haplogroups might interact with pathogenic mtDNA mutations and modify the severity of the pathology of the disease as has been shown with Leber's hereditary optic neuropathy (Taylor and Turnbull, 2005).

1.7.1. Association of mitochondrial DNA haplogroups with diseases

As the mtDNA variants might modulate the replication and transcription of mtDNA (Suissa et al., 2009), several studies suggested that individuals with specific haplogroups may predispose to genetic disorder such type 2 diabetes (Hudson et al., 2014). Leber hereditary optic neuropathy (LHON) is one of these disorders that have been extensively subjected to the investigation (Gómez-Durán et al., 2012). LHON is related to the mutation at nucleotide 11778 in the NADH dehydrogenase gene is more severe among patients in J1c or J2b haplogroups (Carelli et al., 2006).

Another example is seen among East Asian patients where the risk of hearing and visual loss is higher among M7b1'2, individuals, whereas patients within haplogroup M8a are more protected against the LHON disease (Ji et al., 2008). Another example of haplogroup associated with the disease is that HV, U and R0a, HV haplogroups are considered a factor for increased risk of stroke (Abrantes et al., 2016). Finally, sub-Saharan lineages L is found to be associated with an elevation of the risk of asthma (Vergara et al., 2009).

1.8. Y-chromosome

The Y-chromosome, a unique genetic characterisation of male, has ~ 60 million bases of DNA, the majority of which is non-coding and possess the largest part of the non-recombining portion of the human genome (95%) (Novelletto, 2007) Figure 4. These features make it a useful tool to study human migration, male evolution and genetic processes (Underhill and Kivisild, 2007). Chromatin is found in three different parts of the Y-chromosome. The euchromatic region contains the functional genes. It is also the part responsible for the sex-determining part of the Y-chromosome including the sex-determining region Y (SRY) gene. The second region is termed the pseudoautosomal regions (PARs) which are found in the telomeric part of the chromosome. During male meiosis, PARs pair and recombine with the X-chromosome Figure 4. Finally, the heterochromatic part containing the distal Yq corresponding to Yq12. This part is genetically inert and has polymorphic sites that are different in length among male populations. This contains two highly repetitive sequences families, termed DYZ1 which contains 5000 copies and DYZ2, which contains 2000 copies (Jobling et al., 2013a).

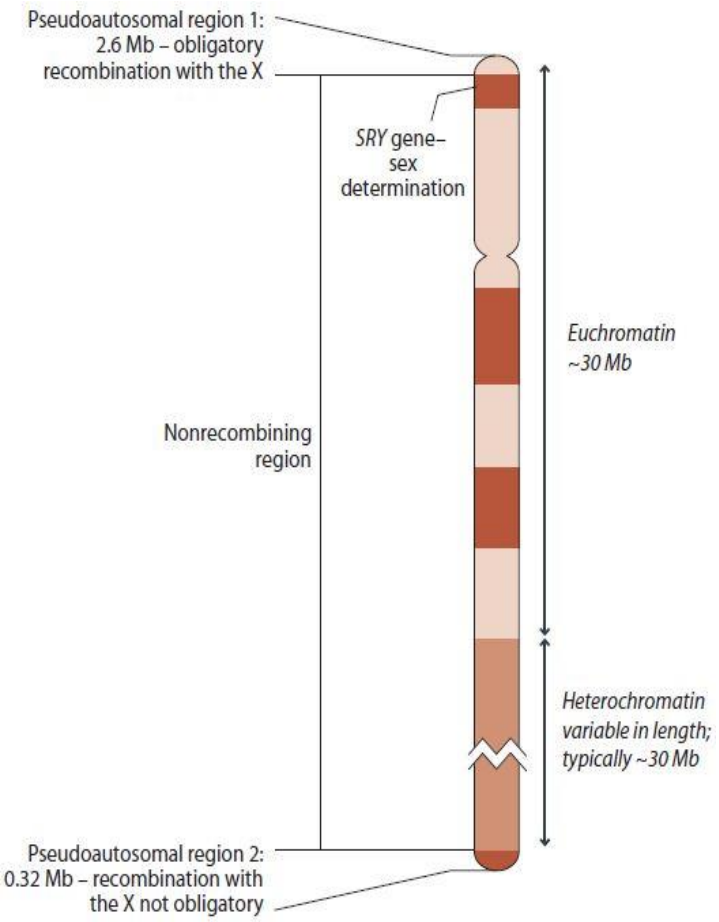

Figure 4. The Y chromosome.
Taken from (Jobling et al., 2013a)

1.8.1. Amelogenin

Amelogenin is an extracellular matrix protein, coded by genes located in both the X- and Y chromosomes exhibits a length polymorphism between X and Y-chromosome copies (Kayser, 2017a). Therefore, the X-Y homologs of the amelogenin gene are suitable for sex determination on the molecular level in forensic casework, archaeological specimens and prenatal diagnosis (Cadenas et al., 2007). The PCR-based sex- test detects a conserved region of the amelogenin in intron one that includes a six bp deletion on the X- chromosome (Kashyap et al., 2006). As a result, in males, two different amplicons differing in size by six bp will be produced from PCR and can be easily distinguished by gel electrophoresis, while in female samples only one product will be produced (Kayser, 2017a).

1.8.2. Y-chromosome mutation

The root of the male-specific region of the Y chromosome (MSY) tree has been assigned as the Y-chromosome Adam. Like mitochondria Eve, Y-Adam is the paternal most recent common ancestor (MRCA) with an estimated time ~142 kya by Cruciani et al., (2011) 200 kya by Francalacci et al., (2013) when they used the mutation rate of the Y SNPs of 1×10^{-9} per base per generation which considered slower than the STRs rate 1×10^{-3} per locus per generation (Pinto et al., 2014). However, when Mendez et al., (2013) utilised the average of rates based on whole-genome sequence data they estimated 6.17×10^{10} position/gamete/year ($4.39 \times 10^{-10} - 7.07 \times 10^{-10}$ per base per year) and obtained a considerably older time of about 338 kya using samples belonging to African American individuals that were found to add an ancient root to the MSY tree Y-chromosome haplogroup nomenclature. (Mendez et al., 2011).

A short tandem repeat (STR) is a repeated nucleotide from 2 to 6 in a specific location within the control region of the chromosome. These repeated nucleotides have a high variation which gives a low match probability among individuals (Buckleton et al., 2005, Butler, 2009).

STRs are highly polymorphic, abundant and distributed along the human genome.

As STRs have multiple alleles, they demonstrate a high level of heterozygosity; therefore, STRs are highly informative for genetic analysis as well as inference of phylogenies in populations (Pinto et al., 2014).

1.8.3. Y-chromosome haplogroup nomenclature

Like mtDNA phylogenies, the MSY phylogeny has a similar nomenclature for haplogroups. The paternal haplogroups are defined by biallelic locus SNPs or indels. In the 1990s, the number of identified binary markers on the Y-chromosome increased to more than 200 (Karafet et al., 2008). Although these markers improved the knowledge of the genetic variation and population history, the inconsistent nomenclature of these binary markers led to confusion (Consortium, 2002). As a result in 2002, the Y-chromosome Consortium (YCC) defined and named the different haplogroups

of Y-SNP according to a system that allowed easy incorporation of newly discovered mutations which lead to unifying the nomenclature (Karafet et al., 2008). In 2008, the YCC nomenclature was updated, and the total number of haplogroups reached 311 with 600 binary markers (Karafet et al., 2008). Currently, the Y haplogroup classification of the male Y-chromosome is used to estimate the population group of the paternal line.

Like mitochondrial DNA haplogroups, Y chromosome haplogroups are divided into letters and then are further divided into subclades, and these levels form a tree Figure 5 (Karafet et al., 2008).

Like mtDNA, the initial splits in the Y chromosome tree were African lineages, haplogroup A, and haplogroup BT. Haplogroup BT is then split into clades B and CT. Similar to mtDNA haplogroup L, Y chromosome haplogroups A and B are restricted to Africa. CT haplogroup includes the majority of African and all out of African chromosomes (Karafet et al., 2008, Cruciani et al., 2011). Haplogroup BCT splits 75 kya into two subhaplogroups, B and CT which include DE. While E is found in Africa, haplogroup D is found in Asia, and haplogroup C is found almost everywhere. Haplogroup K is most frequent in East Asia, where it further splits into N and O haplogroups. Figure 5 illustrates the distribution map of Y-chromosome haplogroups (Underhill and Kivisild, 2007).

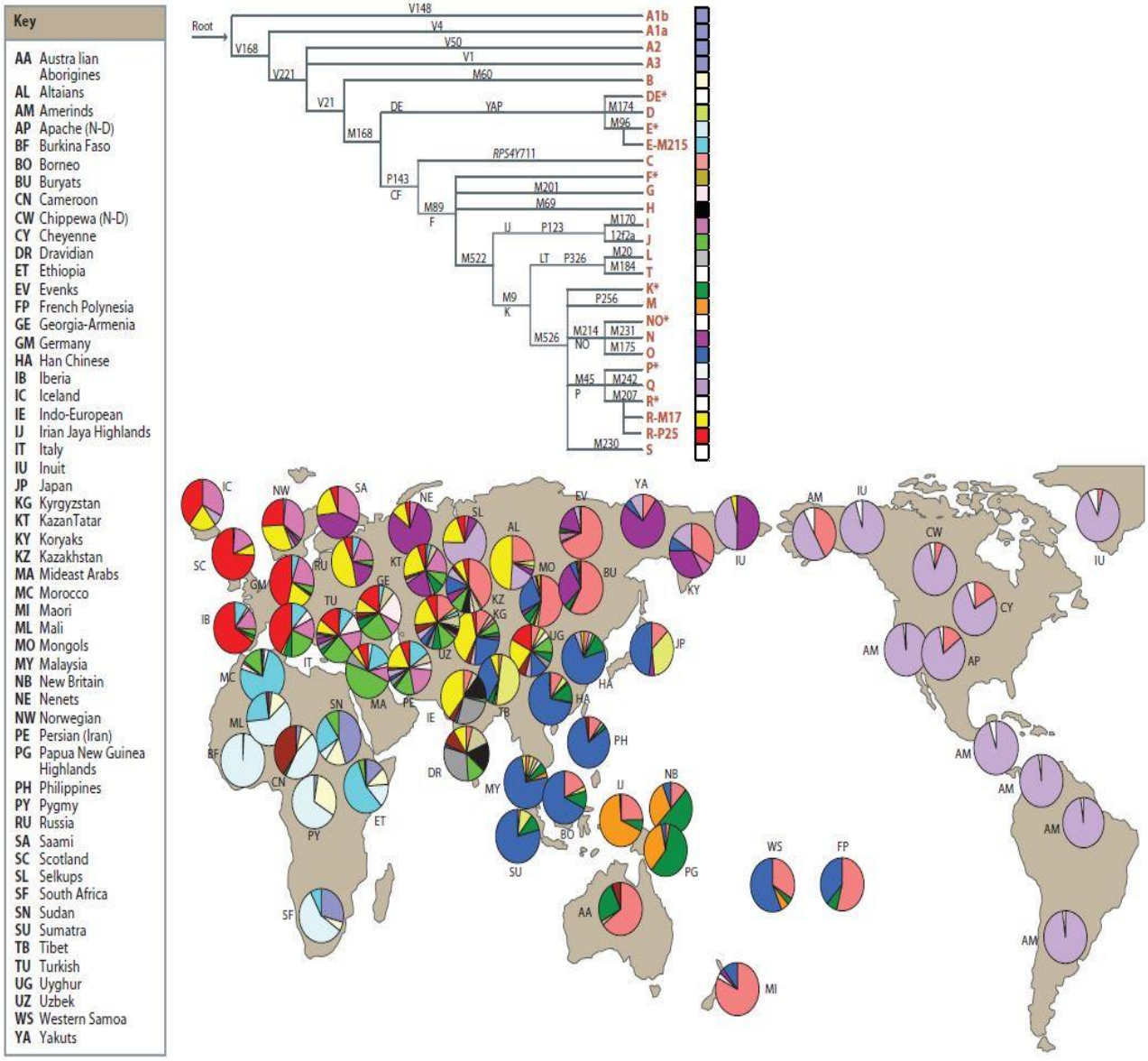

Figure 5. Y-chromosome haplogroup tree and distribution map
(Jobling et al., 2013a)

1.9. Forensic use of Y chromosome

Like a mitochondrial marker, Y-chromosome DNA markers are passed from generation to generation without change unless a mutation occurred along the paternal lineage. As Y-chromosome is only a single allele per individual, it is referred to as a haplotype rather than a genotype. In forensic DNA analysis, the male-specific part of the human Y chromosome is widely used especially if the autosomal DNA profiling is not informative (Kayser, 2017b). Y-chromosomal data is used for determining the sex of the samples found at the crime scene. Y-STRs are applied to

identify the paternal line of the unknown male trace; which is particularly suitable when males and females samples are involved in investigating a sexual assault crime.

Y-STR haplotype analysis is also used in paternity identification of male offspring and other types of paternity testing; this includes identifying victims of mass disaster victims and missing person identification. Furthermore, Y-chromosome polymorphisms can determine paternal bio-geographic ancestry lineage of missing persons in cases where autosomal DNA profiling is uninformative (Kayser, 2017b).

1.9.1. Interpretation of Y-STR results in forensic casework.

In crime scene investigation Y-STR haplotyping can (i) exclude male suspects (ii) identify the paternal lineage (iii) highlight mixed male traces, and (iv) provide information leads for finding an unknown male suspect (Butler, 2009). In the case where the two sequences do not match, then Y-STR helps the exclusion (Kayser, 2017b). Calculation of a random match probability with Y-STR haplotypes is done by estimation of the frequency of the haplotype in the population database (Kayser, 2017b).

1.10. Application of mtDNA and Y chromosome in population genetic studies

Mitochondria and Y chromosome possess several unique characteristics, such as the mode of inheritance, high copy number for mitochondria, the absence of recombination, and a rapid rate of mutations (Jobling et al., 2013a). These features make them a useful tool in the traceback lineages through time and human evolutionary processes without the confusing problems accompanied by recombining nuclear DNA. They also help to understand the demographic history of human populations (Wilson et al., 1995). Mutations occurring in the history of mankind trace the maternal and paternal ancestors, and through this genealogy, it can gain a picture of prehistoric evolution. Sequence polymorphism of mtDNA or Y chromosome can provide magnified information about the genetic diversity among individuals within the same populations and also between different populations (Jobling et al., 2013a). Mitochondrial and Y chromosome studies have supported the

hypothesis that demonstrates Africa is where humans originated from, which had previously come from fossil evidence only (Jobling et al., 2013a). Their analysis has also revealed human migration patterns, including the new World, Europe, and Australia, as well as the Pacific (Pakendorf and Stoneking, 2005). The human population studies using mtDNA analysis showed the variations between the main four ethnic groups (Caucasian, Amerindian, African, and Asian). Variations in mtDNAs have also been revealed populations with a different geographic origin within the same ethnic group (Jobling et al., 2013a).

An example of the power of using mtDNA analysis, a study in the Balearic Islands. Due to the geographical location for the Island in the Western Mediterranean Sea, the Balearic Islands have been settled by several populations migrated from different regions of the Mediterranean (Castro et al., 2010). The study revealed a genetical differentiation of the Ibizan population, with other Balearic populations (Majorca, Minorca and other Spanish and European populations). The differentiation of the Ibizan population may be due to the Carthaginian origin of the Ibizan population and due to inbreeding by the small population and periodical bottleneck and isolation from other Balearic populations (Castro et al., 2010).

1.10.1. Archaeogenetics

In the last few years, the advanced contribution of archaeology and genetics has allowed scientists to reconstruct human migrations. Archaeogenetics is the use of molecular genetics to the study of the history of mankind, particularly through the study of mitochondrial DNA and the Y chromosome (Ottoni et al., 2009) which mainly depend on DNA data from living populations in addition to the contribution from ancient DNA samples. It allows the study of population migration over the world, starting from our roots in Africa back hundreds of thousands of years ago including the recent expansion within a few hundred years (Jobling and Tyler-Smith, 2003, Marchini et al., 2005). Archaeogenetics studies have helped to resolve the ambiguities arising from different disciplines such as anthropology, history and archaeology. This success was achieved initially by

combining the lineages with their geographic distribution. Then the diversity is converted to time depth using the molecular clock. This combining between the two disciplines is termed phylogeography.

1.10.2. Phylogeography

Phylogeography is the analysis of the geographical distribution of the haplogroups in a phylogeny (Jobling and Tyler-Smith, 2003). Since these variants occur at a specific point in space and time, theoretically, each event can be constructed from the distribution. Mathematical formulas can be used to estimate the time and age and distribution of the sequences in higher details and precision and transformed them into a phylogenetic tree especially when complete mtDNAs are used (Soares et al., 2009). It is, therefore, possible to estimate the direction and timing of expansion by analysing the mtDNA lineages from one geographic place to another (Jobling and Tyler-Smith, 2003).

1.10.3. The geographic distribution of lineages

The geographic distribution of lineages can be estimated by analysing samples from several different areas and then identify the similarity in lineages between locations (Macaulay and Richards, 2013).

1.10.4. A gene tree or network

A second element used in phylogeographic analysis provides the hierarchical history of the hypothetical basal lineage and their descendants using a gene tree or network. A phylogeny of the lineages represents a hierarchy of relationships between lineages and establishes hypothetical ancestral lineages from which two or more lineages descend. Without recombination, the variations between lineages will be only be caused by mutations (Macaulay and Richards, 2008b).

1.10.5. The application of a molecular clock

The molecular clock is used in phylogeographic studies to estimate the date of the lineage of the phylogenetic tree (Ho *et al*., 2005, Penny, 2005). At the beginning the mutation rate was considered

linear, however; later on the molecular clock became more refined, meaning that now we use a time-dependent clock, we take into consideration factors like purifying selection and that is what is represents the advantages of the last decade in terms of molecular clock applications (Bromham and Penny, 2003, Kivisild et al., 2006, Soares et al., 2009).

1.10.6. Other complementary lines of evidence

Although the genetic data analysis can show the significance of immigration at a specific point of time and location, no single genetic evidence can be used as an indicator of both the cultural and linguistic affiliation of its carrier (Richards et al., 2000a). There are several other pieces of evidence from other scientific fields that should be used to get a comprehensive picture of demographic occurrence including, historical records, linguistic evidence, archaeological records, climatology and geomorphology, palaeontological data, palaeoclimatological evidence, biogeography, ethnology (cultural and social anthropology), geology and radiocarbon dating (C^{14}) (Jobling et al., 2013b, Gamble, 2015).

1.11. Phylogenetic Trees

Since Andrews et al. (1999) published the revised Cambridge reference sequence (rCRS), rCRS became the reference sequence that use to score the variances present in mtDNA sequences and also used to reconstruct the human mtDNA phylogenetic tree. Phylogenetic trees reflect the history of evolution. Although fossils are necessary evidence to reconstruct species trees. Fossils alone are insufficient and unable to accurately date molecular events (Jobling et al., 2013a).

In contrast DNA data can provide timing to events using molecular clocks. A tree is also a useful tool for the graphical display of distance measures of the relationships of populations. In these cases, it may conclude a model for how diversity arose, but cannot itself represent the mechanism. For example, a tree of populations represents a model whereby populations split from common ancestors (Page and Holmes, 2009).

1.11.1. Phylogenetic trees terminology

Trees are inherently attractive as graphical tools, to displaying relationships between several kinds of entities. These trees have led to introducing partially redundant terminologies that have to be clarified. A tree contains nodes connected by branches or edges between nodes. In a phylogeny, all terminal nodes that are called leaves or operational taxonomic units (OTUs) represent sequence or groups of populations which have data (Page and Holmes, 2009). The aim is to refer groups of populations, known as taxa (singular, taxon) OTUs. These taxa occupy particular nodes, but an internal node that unoccupied by taxa represents hypothetical ancestors (or hypothetical taxonomic units, HTUs). However, other types of evolutionary trees also allow taxa to occupy internal nodes. The ancestors of the sequence are at the root of the tree (Jobling et al., 2013a, Page and Holmes, 2009). Trees can be rooted or unrooted. The tree, where a node that is ancestral to all taxa is called a rooted tree. The root describes the directionality in the tree, based on evolutionary time (Page and Holmes, 2009). An unrooted tree can be rooted by assuming that the root occurs in halfway through the longest branch of the tree (mid-point rooting); however, it is preferably rooted by incorporating an outgroup taxon to all other taxa (Figure 6) (Page and Holmes, 2009, Van Oven and Kayser, 2009). The mitochondrial DNA tree is rooted using Neanderthal, bonobo, chimpanzee and gorilla sequence (Xu and Arnason, 1996, Arnason et al., 1996). The distance between an internal node and the root of the tree determines the relative antiquity of the divergence event it represents. Nodes that are near to the root are more ancient than those far from the root. Unrooted trees are not able to connect the ancestry of different nodes in this way; it is therefore not obvious which nodes are ancestors and which are descendants. The number of possible trees for any given number of taxa increases rapidly. For instance, for nine taxa, there are 135,135 possible unrooted trees, but over 2 million for a rooted one (Page and Holmes, 2009).

The pattern in which the tree is branching is known as topology; also, the descendants of one node, together form what's called a clade. In some trees, when only two branches descended from each

node, this known as bifurcation. On the other hand, some trees allow, more than two branches descend from the same internal node, forming either a polytomy or multifurcation.

A cladogram represents the relationships between taxa and branch lengths between nodes are irrelevant. In contrast, additive trees use branch lengths to show evolutionary distance quantitatively. As a result, additive trees can be different, topologically and quantitatively in the length of their branches. The relationship between a group of taxa and clades allows classifying taxon groupings on the basis of the tree (Page and Holmes, 2009, Van Oven and Kayser, 2009).

Monophyletic is a grouping of taxa that fall into a single clade, which is considered to be a coherent evolutionary lineage; while, paraphyletic is a grouping that excludes other members of the same clade. Also, taxa that span multiple clades are polyphyletic (Figure 6). An important characteristic of trees is that, with the progress of evolutionary time toward the present, branches diverge but do not coalesce.

However, the recombination process can cause lineages to merge while others like parallel mutations cause them to appear to merge. In both cases, the result can be represented as a four-sided closed structure called as a reticulation or cycle. Trees in this structure are known as networks (Page and Holmes, 2009).

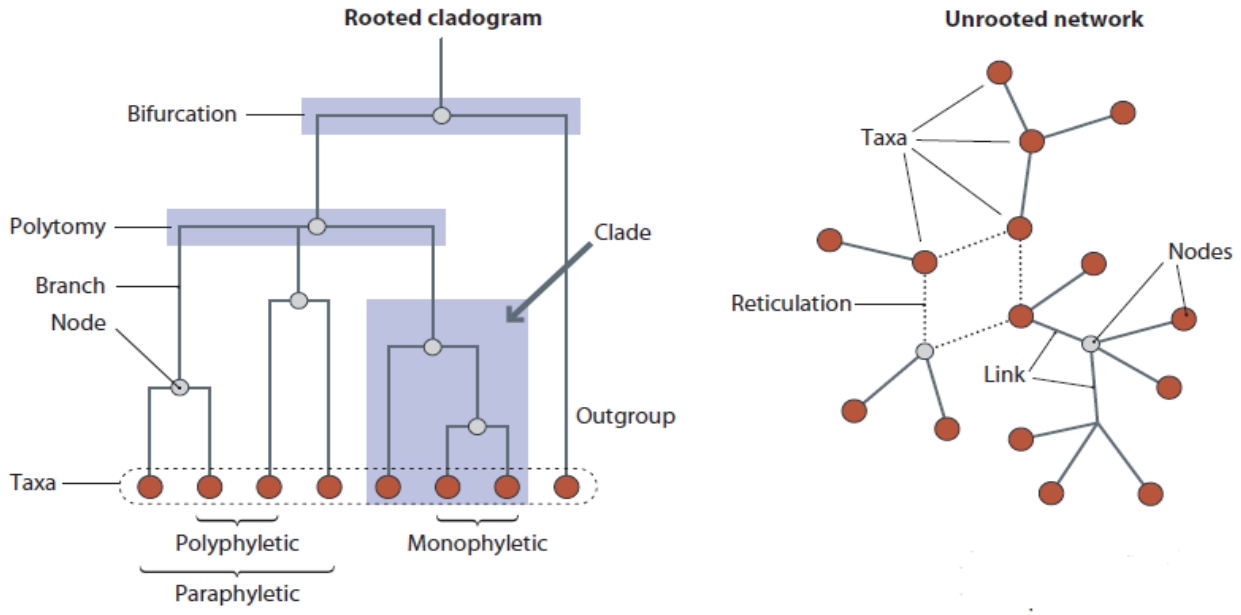

Figure 6. Terminologies for trees and networks
Taken from (Jobling et al., 2013)

1.11.2. Overview of Tree Building Algorithms

Phylogenetic trees can be reconstructed with several evolutionary approaches and not being suitable for all the different types of data available and no single method predominates (Jobling et al., 2013a). The most important construction tree criteria are character-based tree including parsimonious, maximum-likelihood, Bayesian methods and distance methods, including neighbour-joining (NJ) and "unweighted pair-group method (Van de Peer et al., 2009).

1.11.2.1. Distance methods (NJ).

These methods are non-character, the based method for the calculation of genetic distance for pairs of genes. These algorithmic principles strongly decrease the phylogenetic information of the sequences (to one value per sequence pair) (Van de Peer et al., 2009).

1.11.2.2. Maximum parsimonious, MP.

Phylogenetic tree and a multi-alignment can be used to trace back the history by the tree and calculate the minimum number of substitutions that are needed to explore the given data (Van de Peer et al., 2009). MP (Maximum parsimony) is a character-based algorithm that is trying to find the whole tree space that has evolutionary changes. MP algorithm is one of the most favourable approaches due to the assumption that a true tree tends to contain fewer substitutions (Huson et al., 2010). However, these methods face challenges in reconstructing a correct tree when long branches appear. Also, it is not taking into account all possible optimal labellings and homoplasy which may cause the errors (Huson et al., 2010).

1.11.2.3. Maximum likelihood

Maximum likelihood studies try all alternatives to find the best hypothesis as they assume that the best hypothesis that gives the maximum likelihood of the outcome (Huson et al., 2010). Therefore, ML is calculating the likelihood and construct the best tree. ML has the ability to find a correct tree in a relatively short time. ML method is characterised by having the flexibility of models and is the most fruitful method of all the tree-building algorithms. It suggested that ML is the most accurate methods of tree building algorithms (Jobling et al., 2013a).

1.11.2.4. Bayesian method.

The Bayesian method is also a maximum likelihood method; however, it is a character-based method. It performs a probability analysis that allows inferences to be obtained from both the prior Information and data. While ML approach determines the probability of the data (such as the M sequence alignment) given the hypothesis (the tree), a Bayesian approach determines the probability of the hypothesis (the tree) given the data (the sequence alignment) (Jobling et al., 2013a). Bayesian Markov Chain Monte Carlo (MCMC), the model depends on the specified model to finds the one that maximises posterior probability by sampling phylogenetic trees (Huson et al., 2010). The popularity of this method lies in its unparalleled flexibility as it generates trees instead of

calculating the probability of a given tree, complex analytical computations can be avoided (Van de Peer et al., 2009).

1.12. Methods used for DNA sequence variation detection in mtDNA and Y-chromosome

1.12.1. Direct sequencing of the control region of mtDNA

The PCR technique has opened wide horizons of the use of mtDNA genotyping in forensics and archaeogenetics (Wilson et al., 1995). PCR primers are usually used to amplify fragments from position 16,024 to 16,430 for the HVI and 50 to 445 for the HVII region (Wilson et al., 1993). These regions are then directly sequenced by Sanger DNA sequencing (Wilson et al., 1993).

To avoid any false results, most studies sequenced the amplified fragments for both light and heavy strands. Examined sequences are compared with a reference sequence, known as rCRS (Anderson et al., 1981). Any differences between examined and reference sequences such as deletion, insertion and substitutions are considered a polymorphism (Guo et al., 2013). Although the process of sequencing is expensive and time-consuming, it is highly effective and has provided valuable information about mtDNA control region that helps in discriminating between unrelated sequences (Piercy et al., 1993). The first compilation of nucleotide substitution in the HVI and HVII region of mitochondria was constructed in 1996 (Taillon-Miller et al., 1998). Thousands of sequences from HVI and HVII region, from different populations all over the world, have now been analysed (Budowle et al., 1999b, Yang et al., 2014).

1.12.2. Y-chromosome –SNP analysis

Single nucleotide polymorphisms (SNPs) are characterised by a low mutation rate. SNPs are the most widely used binary markers used to reconstruct the history and provide the population haplotype information (Underhill and Kivisild, 2007). These binary markers are single base changes that occur in two possibilities of alleles at a specific position of the DNA sequence. Another form of binary markers are insertions /deletions (indels) events at specific sites on the Y-chromosome

In general, such binary markers are an important tool in the identification of deep splits in the Y-chromosome line, and they represent unique event polymorphisms in human evolution (Francalacci et al., 2013).

1.12.3. Y-Short tandem repeats (Y-STRs) analysis

A short tandem repeat (STR) is a repeated nucleotide from 2 to 6 in a specific location within the control region of the chromosome. These repeated nucleotides have a high variation which gives a low match probability among individuals (Buckleton et al., 2005, Butler, 2009). In forensics, four nucleotide repeats are used for genetic fingerprinting of individuals analysis. Capillary electrophoresis is used to determine the number of repeats in each locus by using fluorescent dyes. If only one peak appears in a particular locus, that is called a homozygote, while if two peaks, this indicates a heterozygote (Figure 7) (Buckleton et al., 2005, Butler, 2005, Butler, 2009).

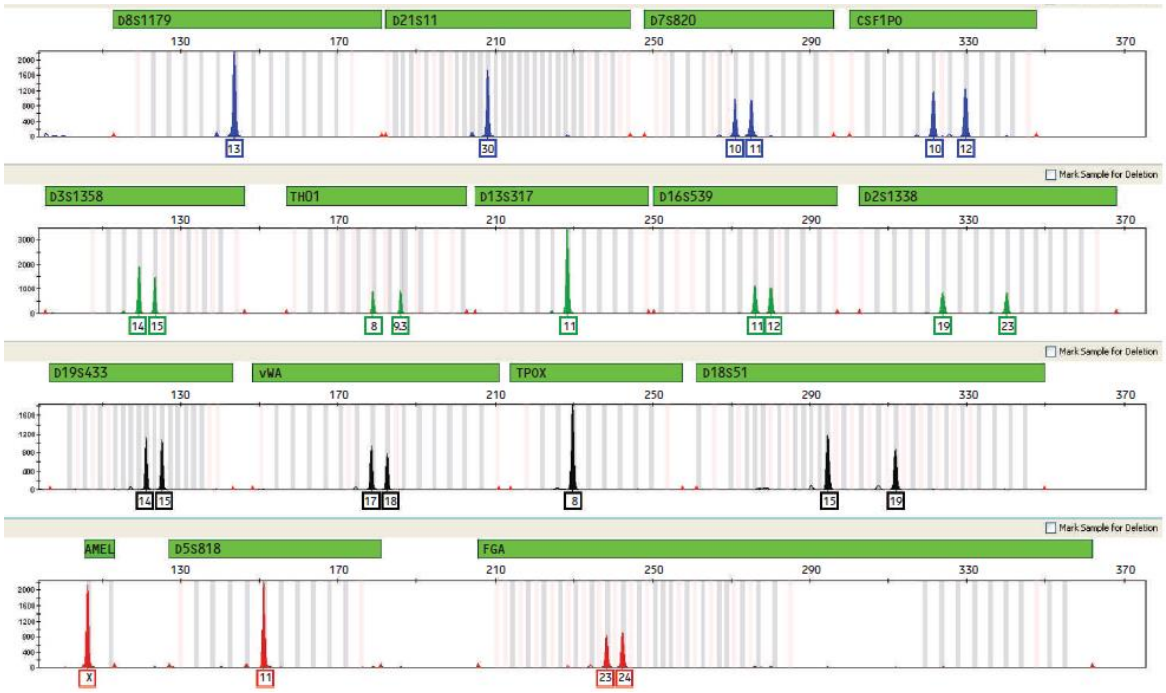

Figure 7. A high-resolution microsatellite multiplex for DNA profiling

Taken from (Jobling, 2013)

An example of a DNA profile generated using Applied Biosystem, a PCR multiplex run on a capillary electrophoresis unit. Each peak is a PCR product and is labelled with the allele call (representing the number of repeats), one peak indicates of homozygote alleles, while two peaks indicate of heterozygote alleles, the size of the fragment (in bases), and the peak height (in relative fluorescence units). This individual is a female, as seen by the only "X" peak at the amelogenin position

The STRs, have a higher mutation rate than other parts of the Y chromosome. It is also, highly variable among a male population making Y-STR an exceptional tool to track the recent genetic events (Butler, 2009). Y-STRs make excellent genetic markers because (i) they are passed through the paternal lineage and (ii) the lack recombination in meiosis stage. These features made Y-STR an excellent tool that can be used to define the descending order of all human non-recombining Y-chromosome (NRY) polymorphism and to conclude the order and time of their descent within the phylogenetic tree (Butler, 2009). Moreover, the NRY is considered one of the best parts of the human genome for population studies as it provides a strong genetic differentiation over geographic distance (Butler, 2009). It is therefore useful for reconstructing human population history, including estimation of demographic parameters and is also useful for forensic application such as genealogical relationships and male lineage determination (Van Geystelen et al., 2013).

1.12.4. Next-generation sequencing

High throughout Next-generation sequencing (HT-NGS) technologies are revolutionizing the human genome research in the relatively short time since 2005 (Pareek et al., 2011). Next-generation sequencing (NGS) technology, with its high-throughput capacity and low cost, offered a new opportunity in the genetic studies emerge by harnessing the power of NGS technology, which can be applied to simultaneously analyze multiple loci in different genetic contexts, such as autosomes, mitochondrial and Y chromosomes (Qian et al., 2017). NGS techniques greatly assist to shift from analysing few reliable long mtDNA reads 800 bp (usually HVR of mtDNA in Sanger sequencing to thousand reads (whole mtDNA genome) in Next Generation Sequencing (NGS) requires new computational models and additional attention interpreting results. Like mtDNA, NGS also has several advantages for Y-STR analysis include high throughput, low cost, simultaneous detection of large numbers of STR loci (Yang et al., 2014). However, analysis of large sequence data is complex, and challenges lie in the consequences of misinterpretations data (Underhill and Kivisild, 2007).

1.13. Genetic variations and origin of modern Humans

1.13.1. Human Variation

The contemporary genetic variation in the human is the result of an intricate evolutionary process, which took place all over the world and for the hundreds of thousands of years. These variations occurred through mutations, genetic drift process, gene flow and natural selection (Marks, 2001). Analysis of genetic variation in modern human enables a deeper understanding of both human history and human diseases, which are integral because both have affected by different environments including pathogens, climates, diets and other selective pressures. All of these factors have influenced migration patterns and variation in population size, which finally affect the human distribution and the human population's diversity over the world as well as the distribution of diseases among modern humans. Consequently, a concurrent focus on both population history and diseases provides insight to understand many processes that participate in the genetic diversity of mankind. (Balaresque et al., 2007).

1.13.2. Mitochondrial Eve

Mitochondrial Eve is the sequence that represents a theoretical ancestor to all remaining mitochondrial lineages in the human species. However, the different parts of the genome have different histories. While the age of the mitochondrial Eve or the coalescence time of the human mitochondrial tree is 160 kya according to Kivisild's synonymous rate (Kivisild et al., 2006), and according to the Mishmar coding-region rate, the age of about 200 kya (Mishmar et al., 2003) Figure 8.

From the root, changes in mitochondria sequence lead to a split from haplogroup L0 to all of the rest of haplogroups both within Africa and outside Africa, change at position 1048 which is a T in L0 and a C in the other haplogroups, the presence of a C in the Neanderthal sequence makes this state more probable in the root of the human tree. Also, the position 10589 presents an A in L0 and a G in the rest of the tree, and the presence of an A in the Neanderthal sequence indicating that this

was probably the state in the root. The other changes in the tree are position 182 is a C in L0 and a T in the rest, the position 10664 is a T in L0 and a C in the others, the position 10915 has a C in L0 and a T in the rest of the tree. The position 12007 in the mitochondrial Eve also seems well defined with an A in L0, a G in the remainder of the G human tree, the position 13276 is a G in L0 and an A in the others, and the position 16230 is a G in L0 and an A in the rest of the human tree. The position 16230 is a G in L0 and an A in the remainder of the tree. The consensus would indicate that the mitochondrial Eve presented a G. However the Neanderthal sequence presents an A in this position. (Behar et al., 2008, Torroni et al., 2006, Maca-Meyer et al., 2001, Bensasson et al., 2001).

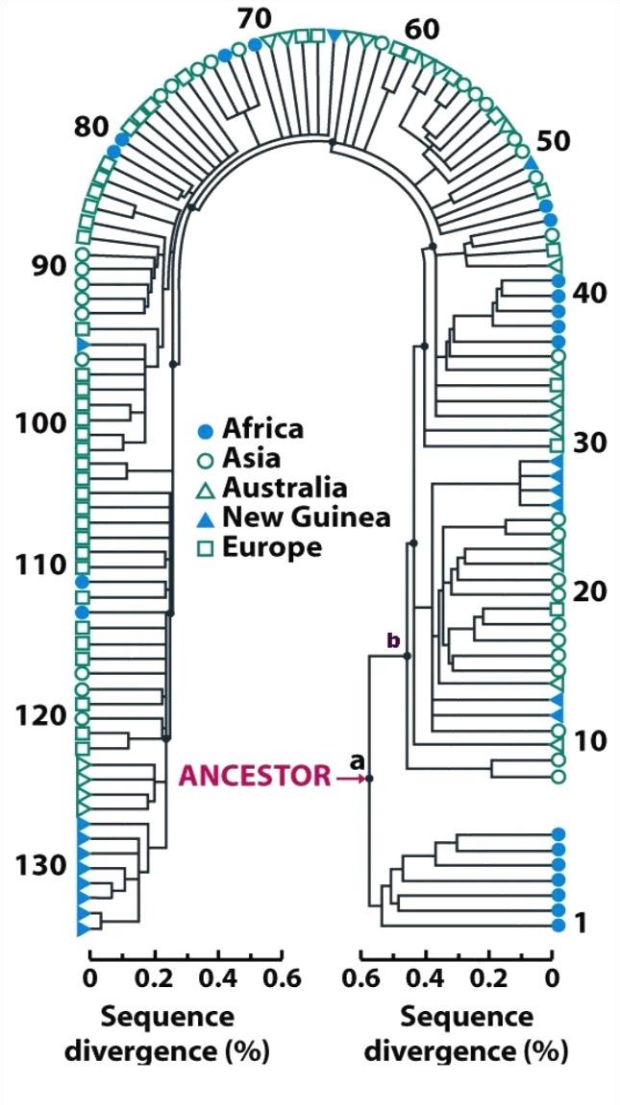

Figure 8.A tree representing mtDNA sequence relationships among modern humans (Cann et al. 1987).

1.13.3. Y-chromosomal Adam

The most recent ancestor of all male human or the most ancient male whose Y chromosomal lineage can be traced without interruption up to the present called Y-chromosomal Adam. Y-chromosomal Adam lived in Africa roughly between 120,000 to 156,000 years ago. The first branching in the MSY tree has separated the African clade A from clade BT, whereas the second branching determines the subdivision of BT in clades B, mostly African, and CT, which comprises the majority of African and all non-African chromosomes (Cruciani et al., 2011).

1.13.4. The Origin of Anatomically Modern Humans (AMHs)

There are several factors that may hinder the recovery of archaeological evidence, including for example a ~80-meter rise of sea level that leads to submerging the evidence under the sea (Metspalu et al., 2006) and the tectonic movements of the continental shelves (Chen and Zhang, 1991, Klein, 2009). Also the constant dispute about the accuracy of fossil dating techniques. In addition, the poor DNA quantity and quality in hominin fossils older than 100 kay makes them impossible to analyse. However, the Neanderthals fossils still contain endogenous DNA that provides good genetic data (Goodwin and Ovchinnikov, 2006). There are two hypotheses have been raised to explain the emergence of anatomically modern human (AMH), the first one is the "multiregional" hypothesis " and the second is "Out of Africa hypothesis''.

1.13.5. Multiregional evolution hypothesis

According to the multiregional hypothesis, *Homo erectus* populations, migrated out of Africa to the various regions of the world more than 1 MYA (Nei, 1995) and the AMH developed from *Homo erectus* to *Homo sapiens* in a number of areas of the Old World (Africa, Asia and Europe) with different modern human characteristics arising at different times in different places (Jobling, 2013). The parallel development from *Homo erectus* to *Homo sapiens* led to homogenizing the differences between them (Wolpoff et al., 1988). This model suggests that our ancestors lived on several continents in the past. This hypothesis was supported by the similarity between modern humans and

Neanderthals where they found 16 morphological similarities shared between them suggesting that the source of this gene in AMHis the Neanderthals (Wolpoff and Thorne, 1991). In contrast, there is no evidence of similarity in other species of other animals on different continents (Ayala, 1995).

1.13.6. Out of Africa hypothesis

The oldest human remains (cranial remains) were found in southern Ethiopia, dated to 198 kya (McDougall et al., 2005). Human dental remains have been found in South Africa, dating between 80 to 100 kay (Grine and Henshilwood, 2002). However, it is hard to know the origin of AMH within Africa (Deacon, 1992, Deacon and Geleijnse, 1988). Ancient mtDNA analysis seems to support the out of Africa hypothesis, where the analysis of the Neanderthal mitochondrial control-region (Ovchinnikov et al., 2000) revealed that both AMH and the Neanderthal evolved separately (Krings et al., 1999).

Cruciani et al. (2011) have obtained an age of 142 kya for the coalescence time of the revised MSY tree. Also, another evidence for an African origin for the human is the number of deep branchings leading to African-specific clades has doubled. Also, analysis of 2204 African DNA samples revealed that the deepest clades of the MSY phylogeny are found in Africa, opening new perspectives on the early human presence in the continent.

With the advancement of new sequencing technologies like NGS, several complete ancient mtDNAs were sequenced, and the divergence was estimated to be between 511 and 550 kya (Briggs et al., 2009, Soares et al., 2009). The two oldest branches of the tree are L0, and the remaining mtDNA lineages are five major binary splits. All of these splits occur before the out Africa. The L0d and L0k, L3, L4, L5 and L6 represented an Africa haplogroup (Macaulay and Richards, 2008a). The two main Non-African haplogroups, M and N, migrated from Africa 60 kya. (Soares et al., 2009). These lineages are a split between L1 and the remaining human lineages and then followed by the separation of L2, L5, L6, and finally the separation of L4 and L, Figure 9. (Torroni et al., 2006, Soares et al., 2009, Briggs et al., 2009). Mitochondrial Eve, who is the most recent

common coalescent ancestor, lies at the root of, L0 mtDNA haplogroups L0 to L6 contains the oldest branches of the mtDNA tree and have been found in sub-Saharan African populations; this gave further evidence of an African origin for *Homo sapiens* mtDNA at ~200 kya. All non-African mtDNA haplogroup L3 dispersed from East Africa 60 kya then diverged into haplogroups M and N which constitute all of the non-African diversity (Jarman et al., 1986, Forster, 2004). The two more recent out-of-Africa founder haplogroups have less than one-third of the diversity and have coalesced much more compared with the African mitochondrial Eve. Haplogroups M and N are separated from the root of L3 by four and five mutations, respectively (Maca-Meyer et al., 2001, Macaulay et al., 2005, Torroni et al., 2006). The Mishmar rate dated L3 to around 80 kya (Mishmar et al., 2003, Torroni et al., 2006) and 65 kya years using the synonymous rate (Kivisild et al., 2006) Figure 9. Using a Maximum likelihood approach, Soares et al., (2009) calibrated the molecular clock using the whole mtDNA genome and obtained an estimation age of the L3 at ~70 kya. M and N that are Non-African sub-lineages radiated towards Asia and a small subset of N lineages colonised Eurasia and Europe. M and N haplogroups dated to 65-60 kya Gounder Palanichamy et al., (2004), Macaulay et al., (2005) using the Mishmar rate and whereas 45–50 kya using the synonymous rate (Kivisild et al., 2006). There are two hypotheses regarding how many lineages that are visible today left Africa in the exodus. The first suggests that an L3 root type sequence went out of Africa and evolved into M and N outside of Africa, while the second hypothesis is that the two lineages evolved in Africa then left Africa, corresponding to the M root type or one pre-M lineage and an N root type or one pre-N lineage (Kivisild et al., 2006).

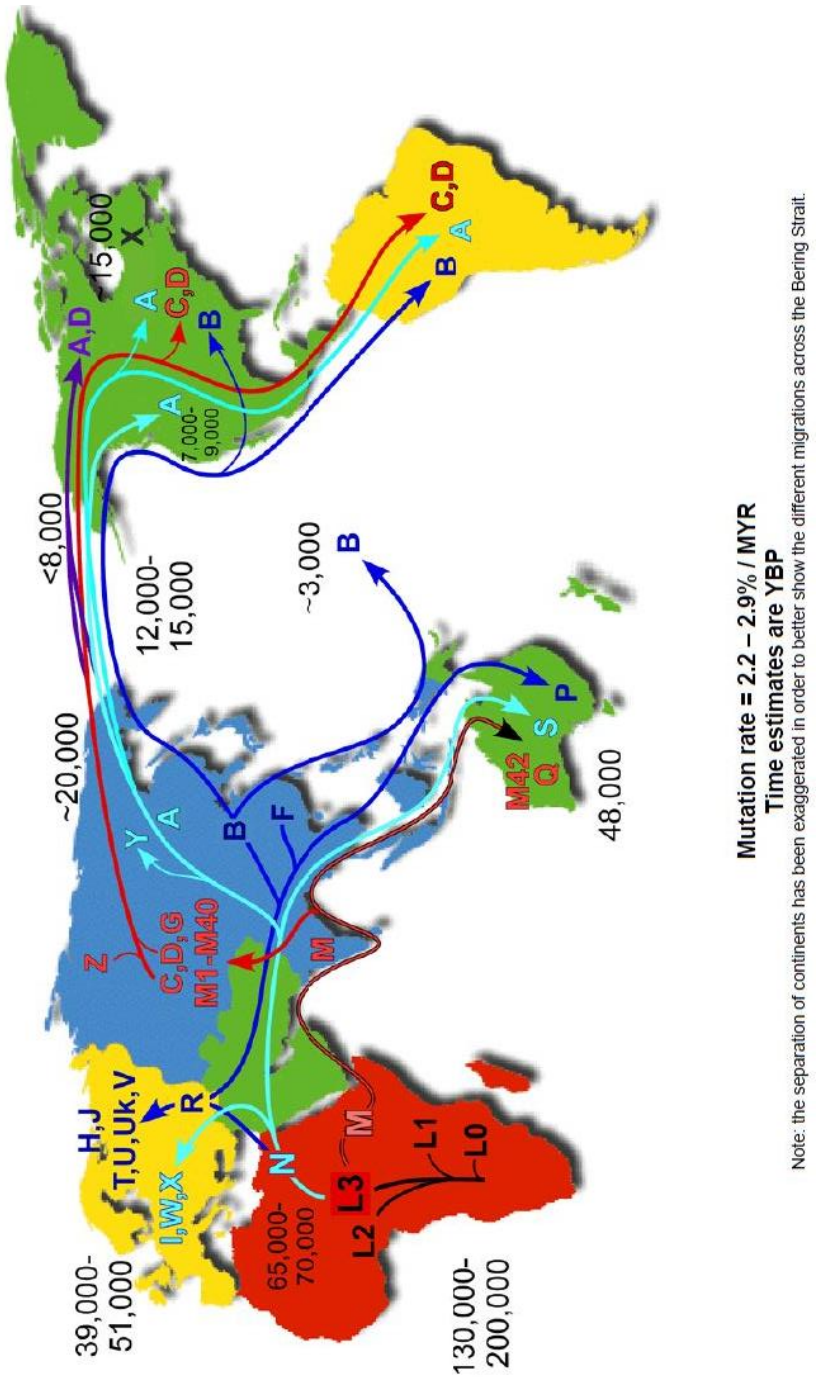

Figure 9. mt DNA haplogroup migration patterns.

Taken from **http://www.mitomap.org/pub/MITOMAP/MitomapFigures/WorldMigrations2013.pdf**

1.13.7. The Southern Route

It is known that the mitochondrial lineages outside Africa descend from two lineages labelled M and N. A suggested model about the route of out Africa migration is the Southern coastal route. A

suggested model about the route out of Africa migration is the southern coastal route to toward southern Arabia via the Red Sea. This is mainly due to the presence of Neolithic lineages such as R0a and J1b in the area. However, the absence of M and N haplogroups indicates that southern Arabia has been more a receptor of human migrations, than an expansion region (Abu-Amero et al., 2008).

1.14. Origin and migrations of the North Africa populations

North Africa region has a complex history of demographic events that is different from the rest of the continent where the North African history is limited to an east-west direction due to the Mediterranean Sea and the Sahara Desert barriers (Fadhlaoui- Zid et al., 2011a). During history, North Africa was subject to invasion and settlements of the different populations including the Phoenicians, Romans, Vandals and Europeans. All of these populations have therefore contributed to the current genetic variation in the area (Cherni et al., 2011). According to the historical studies, modern *Homo sapiens* inhabited North Africa since Upper Palaeolithic times (Newman, 1997), and the first known Palaeolithic industry, was the Aterian at ~45 kya (Garcea and Giraudi, 2006). mtDNA analysis showed that the population entrance into the Levant was earlier than 50 kya, that paved the way to the dispersal of modern humans north-westward into Europe and in the same time south-westward into Northern Africa (Newman, 1995). The second defined technology in North Africa was Iberomaurusian between 22 and 9 kya. Analysis of Iberomaurusian remains from the Afalou in Algeria and from Morocco shows North African and Eurasian components aged to 22 kya (Newman, 1995). A very low contribution of a sub-Saharan African component was also found in the Iberomaurusian samples pointing to a genetic flow between Southern and Northern North Africa (Kefi et al., 2018). Furthermore, it is said that the European Mesolithic Tardenoisian culture had its parallel, in the Upper Capsian of North Africa (Bosch et al., 1997). During the Last Glacial Maximum, the climate in the warmer regions of the world was cooler and drier, and the Sahara spread into Northern Africa. At that time refugial areas were concentrated as the Franco-Cantabrian

and Balkans refuges in Europe and Levant. This period then followed by Bølling warming in the postglacial period that allows the re-expansion processes to begin and resettlement of Central and Northern Europe. These events are evident by mtDNA analysis, where haplogroups H1, H3 and V contributed to the gradual re-peopling of Europe from the Franco-Cantabrian refuge in the postglacial period (Ottoni et al., 2010). Presence of these haplogroups in North Africa is evidence of trans-Mediterranean contacts between Northern Africa and Western Europe. The high incidence of H1 and H3 in western parts reveals a possible link with the postglacial expansion from the Iberian Peninsula Strait of Gibraltar, into North Africa. The archaeological record also showed a Mesolithic culture, the Capsian industry (10 – 4.7 kya) (Desanges, 1990), that involved farming and agriculture spreading in North Africa, which began around 5.5 kya (Cherni et al., 2011). Analysis of Y chromosome lineages in North Africa showed a high frequency of a Neolithic origin specific North African haplogroups E1b1b1 (Arredi et al., 2004; Cruciani et al., 2004, 2007, 2010; Semino et al. 2004).

Despite the importance of sub-Saharan lineages contribution, the majority of lineages in North Africa are of Eurasian origin (Rando et al., 1998; Krings et al., 1999; Plaza et al., 2003; Fadhlaoui-Zid et al., 2004; Harich et al., 2010). The earlier arriving of these lineages were the Paleolithic haplogroups U6, M1b, which are almost specific of northern African populations. The remaining lineage arrived at the end of the Last Glacial Maximum that expanded from Levant refuge such as R0a, J1b, U3 and from the Franco-Cantabrian refuge such as haplogroups H1, H3, U5 and V (Maca-Meyer et al., 2003; Olivieri et al., 2006; Gonzalez et al., 2007; Cherni et al., 2009; Ennafaa et al., 2009; Rhouda et al., 2009; Ottoni et al., 2010).

As the focus of the research described in this thesis is the Libyan population, the following sub-chapter focus on this population.

1.15. Libya

1.15.1. Historical Background of Libya

Libya (Figure 10) is a North African country, with an area of 1.7 million km^2, which occupies a strategic geographic location on the Mediterranean, the junction point between Africa and Europe. Therefore, it has been a place of multiple civilisations and a centre of contact for many population migrations in the successive historical periods (Najem, 2004). The complex interaction between geographic and climate factors have also been pivotal in the geographical diversification that is currently the human population in Libya (Garcea and Giraudi, 2006, Hassan, 1975).

Figure 10. The geographic location of the main cities in Libya
from (Webster, 2012)

For over three millennia Libya was occupied by a series of peoples who, to a greater or lesser extent, left behind rich legacies in terms of the scale and importance of historic sites and, in more recent times, notable examples of architecture (Falola et al., 2012, Hassan, 1975, Pereira et al., 2009). The name Libya is of a Greek origin from "Libou," which is the ancient name of native Berber (Amazigh) tribe. Libya was first inhabited by Amazigh, which was followed by Phoenicians, Greeks, Romans, Arabs and Ottomans before it became independent in 1951 after a period as an Italian colony (Blunsum, 1968). The Phoenicians established settlements during the ~2.7 kya in Sabratha, Tripoli (Oea), Libda (Leptis Magna), Macomades Euphranta and Mdinat Sultan (Charax). However, unlike later occupations, little visual evidence of their settlement has been discovered (Ennafaa et al., 2009).

The Greeks, who coincided with the Phoenicians, largely concentrated their attention on the eastern part of the country. They established five cities that formed the federation known as the 'Pentapolis' - Cyrene, Teuchira, Berenice, Apollonia and Al-Marj. (St John, 2012). With the passing of the Ptolemaic Kingdom into Roman control, Cyrenaica became a Roman province (~2.1 kya). About the same time, following the defeat of the Carthaginians, the Roman influence spread westwards and eventually the entire coastal zone came under their control. Magnificent cities were constructed in Leptis Magna and Sabratha. By the ~1.6 kya, the coastal zone of Libya had become Christianised. A brief occupation by the Vandals gave way to the control by Byzantium based in Constantinople (Istanbul) (St John, 2012). By the ~1.3 kya, a movement occurred that, subsequently has had a monumental and lasting impact on Libya. With the Arab conquest, the Islamic religion was established. The first Arab expansion started in Egypt in ~1.3 kya. This migration started with a few thousand individuals that spread language and religion over to the Berber. (Ennafaa et al., 2009). After brief incursions by some European powers (Norman Sicilians, the Spanish and the Maltese (Order the Knights of St. John) much of North Africa region fell under the control of the Ottoman Empire largely through a series of semi-autonomous rulers, particularly

the powerful Karamanli dynasty (1711-1835). In 1835, Tripoli came under the direct rule of Turkey. In 1911, Libya was then invaded by the Italians, and after World War II, the country came under joint British and French administration until the granting of independence by the newly formed United Nations in December 1951 (Fushaika, 1962).

1.15.2. Population geography and climate

Based on the most recent census in 2018 the current population of Libya is 7.2 million (Wikipedia) and the population is a mixture of several races that originated through the historical migrations, with most of them migrating from the Arabian Peninsula in the 7th century AD, when Arab Muslims conquered North Africa (Triki-Fendri et al., 2013).

By the end of the 10th century, when there was a big movement of the Arab populations (mostly Bedouins) into North Africa the only populations groups that escaped the Arab influence were some the tribes that escaped to the Nafusa mountains or settled in isolated cities such as Zuwara in the north, Ghadames in the southwest and Oujla in the east (Fadhlaoui-Zid et al., 2011a, Najem, 2004). In the present day, the majority of the Libyan population (65%) lives in Tripolitania, more than 30% live in the Cyrenaica province while the remaining of inhabitants (5%) live in the Fezzan area of the Sahara.

Several distinct ethnic groups constitute the present population of Libya, most of them are Arab, although there has been intercultural marriage with the Berbers and other local peoples, for centuries, that may have produced miscegenation of peoples. The Arabic-speaking Muslims, with ancient Arab and Amazigh, represent 90% of the country's population. Tuaregs, Toubu, Greek, Turkish and other black African groups make up for the rest of the population (Academia, DESA, 2011).

Present day Libya is a large country possessing a surface area of 1.7 million sq. km, with over 90% covered with desert. The Mediterranean flanks Libya to the north, Algeria and Tunisia to the west, Niger and Chad, Sudan to the south and Egypt to the east.

The climate of Libya is characterised as being arid with different ranges of temperatures. Northern areas or coastal regions, in general, are temperate with a winter rainfall of 35 cm, although some areas experience frost, temperature does not fall below 5 °C in winter while summer temperatures do not exceed 30 °C (Gentilli, 1955, Roffa and Farhat, 2014). In the south, the climate is extremely hot with little or no rain. No permanent rivers exist, but normally dry river beds (wadis) flood during rain and remain so for a few days. A hot, dry wind from the south (gibli), which can rapidly raise the temperature in the north, usually blows, for a day or two at a time, during the spring and autumn although it may rise suddenly at any time. The Mediterranean Sea in the north and the Sahara desert in the south are the primary factors that determine the climate in this region, and have had a major impact on the distribution of the population as well as the location of agricultural and commercial development (Gentilli, 1955, Hassan, 1975, Roffa and Farhat, 2014).

1.15.3. Socio-political history and linguistic background

Arabic language and culture were first introduced into Libya from the seventh to eleventh centuries, by the Arab Muslims, who migrated to Libya. However, the local people now all claim to be called Arabs (Najem, 2004). Thus, the current Arab population is a combination of several different groups. As intermarriages between Arab and indigenous people (Berber) have taken place over many centuries, it became difficult to distinguish between Arabs and Berber. As a result, a mixed race of Berber ancestry with the Arabic-speaking Muslims makes up about 90% of the current population of Libya. The remaining are Berbers, Tuareg, Tebou, black Africans and small groups of Greeks, Armenians, Muslim Cretans and Maltese (Holes, 2004). In order to analyse the language situation in North Africa in general, it is essential to understand the geographical fabric, linguistic contact between Berber and other existing languages. Also, it is crucial to investigate the contact between the Imazighen, the autochthonous inhabitants of North Africa and other new settlers of different ethnic background. These demographic and linguistic effects provide fertile ground for cultures and language conflict which lead to conflict identities. Therefore, an overview of the

relation between the socio-political history of the people and their linguistic characteristics might help to understand these issues fully. Libya possesses geographical position between the eastern and western basins of the Mediterranean, which has led to a complicated speech community structure throughout history and moved through several phases of linguistic complexity. However, it is difficult to find any record to explain the extent to which all these languages interacted with each other. However, one thing is clear the survival of the Berber and their language in the face of waves of occupation has probably been driven by political and social factors. When the Arabs arrived, few people in the larger cities spoke Imazighen. During the Romans period, most people in the towns and cities spoke only Latin, and many individuals had been bilingual or trilingual. With the 11th century A.D., the Berber populations convert to Islam changed forever the language situation in North Africa (Najem, 2004). The cultural and religious effects favoured the Arabic language so that by the time the Turks arrived they made Libya one of their countries among many other Arabic-speaking countries in the mid-14th century. Berber was replaced by Arabic as the language of the majority whereas Turkish later became the language of the court (Najem, 2004).

1.15.4. Libyan Berber or the Imazighen

The Berbers call themselves Imazighen, which means free and a nobleman. Berber is a Greek derivative referring to those who spoke a language other than the Greeks (Kossmann, 2013).

The remaining of Imazighen language appears now in the form of the Berber dialects or Imazighen language still spoken by some populations of North Africa such as the Shults in Morocco, the Kabyles in Algeria, the Tuaregs in the Sahara Desert, the Tunisian Berbers and the Libyan Berbers (Černý et al., 2007). Berbers inhabit sporadic areas in North Africa, from the coast to the Siwa oasis in Egypt to the Western Moroccan coast, and from Tripoli in Libya and Tunisia in the north to the mid-Sahara. Zuwura is the only coastal Berber city in Libya while the vast majority of Berbers live in Jabal Nafusa. Some Berber also inhabit the oasis of Awjila in the south-east of Libya and Ghadames in the south-west. Whilst to Tuareg Berber live in West Libyan desert area, Figure 11

(Chaabani and Cox, 1988). The origin of the Imazighen is not clearly known, Imazighen may be the descendants of Mesolithic Capsian populations, or of the later Neolithic people who moved from the Middle East via Egypt. Although, Imazighen experienced a complicated history with many invasions and migrations by Phoenicians, Vandals, Romans and Byzantines, their genetic effect on the Berber population seems very limited. In contrast, the most significant genetic contribution to Berber populations was by the Arab conquest (Coudray et al., 2009).

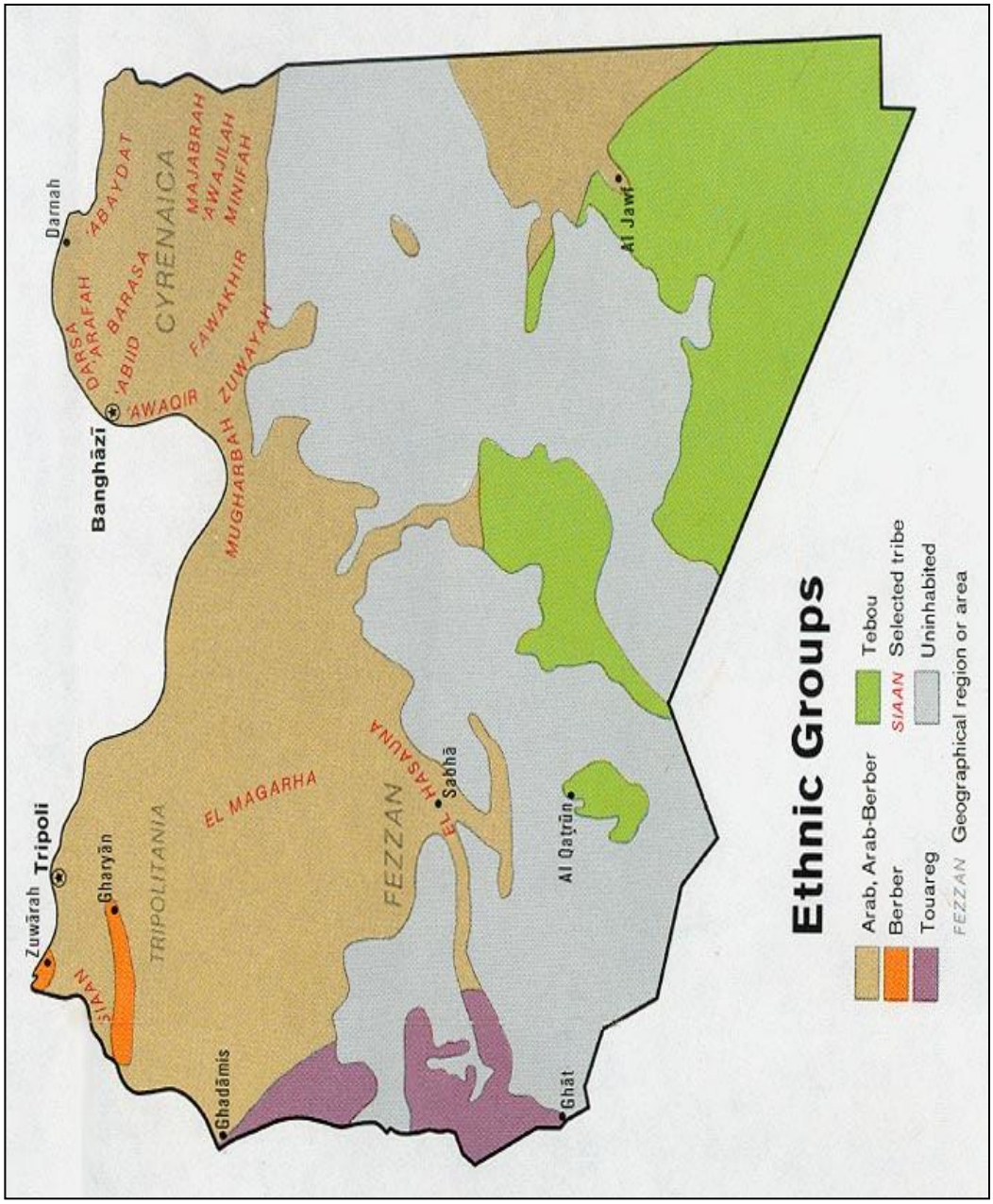

Figure 11. Ethnic group distribution in Libya

Nafusa mountain indicated by larger orange area. (Carment, 2012)

1.15.4.1. The origin of Imazighen

There have been huge confusions about the origin of Imazighen, where several opinions have raised. The first opinion considers them to be the ancestor of the ancient Arabs. Thus, they migrated from the peninsula, especially the Fertile Crescent to the West of North Africa, where they were subjected to environmental changes and ecological calamities (Najem, 2004). The second opinion says that Berbers are from Hindu-European origins that migrated from India, through the Persians and the Caucasus to distribute throughout Europe, and finally crossed the Mediterranean from the Iberian Peninsula to dominate North Africa (Najem, 2004). Others suggested that Berbers are of European ancestry origin from tribes of Finland, Sweden or "Germanic" Vandal descent especially those that are blue-eyed and white-skinned (Najem, 2004). The fourth opinion has prophesied that the Imazighen are the offspring of Ham, the son of Noah. Finally, it also has also been suggested that the Imazighen are a combination of different races living around the Mediterranean, including the Phoenician, Liby, Egyptian, Etruscan, Canaanite, Sicilian, Sardinian, Greek and Iberian (Najem, 2004). The Berbers are divided into the town dwellers (urbanised) and the Bedouin (nomads) urbanised live in towns near the coast and infertile mountains. The Berbers are found in the whole of North Africa and the Sahara. The Berbers in Libya are found mostly in the North to a lesser extent in the west oasis and the south in the desert (Nelson and Harold 1979). After the Islam arrived in Libya, the Imazighen and Arab have interacted to change the demographic and geographic map so that it is almost impossible to distinguish between the Arab and Berber. It is also very difficult to count the Berbers who came to live or moved around the area during different periods of history (Najem, 2004). Despite this, the Berbers of Libya have succeeded in preserving their culture and language by living in isolation that has helped them to keep their identity. It is clear that the Imazighen in the East of Libya has almost no traces apart from a few names, and most clues about Imazighen history in Cyrenaica have disappeared. Also, Awjila and Ghadames populations doubt their affiliation to the Imazighen in contrary to their brethren in the north of Libya (Nelson and Harold 1979). One of the most important regions that Berbers now live in is

Jabal Nafusa sometimes called Jabal al-Garbi, which contains the cities of Nalut, Yifrin, Jadu, Kabaow. Another population inhabits the coastal city of Zuwara (Najem, 2004).

1.15.4.2. Libyan Berber Language and tribes

Berber is one of the branches of the Afro-Asiatic language family, also known as Hamito-Semitic which includes Chadic, Cushitic, Ancient Egyptian, and Semitic. Arabic is a part of the Semitic branch which presents some similarities with the Berber language (Kossmann, 2013). There are many Berber dialects spoken in present-day Libya as outlined below:

Djebel Nafusa dialect.

The Djebel Nafusa dialect is spoken by ~200 kya people living in a western mountain in Libya. The Nafusa dialect has many features in common with Zenatic dialects, but also, with Ghadames and the Libyan-Egyptian oasis Siwa dialects (Kossmann, 2013). Nafusa mountain Berber inhabit the area from Tagoura and the Tripoli coast to Sabratha in the West. Then, they were forced south toward the western mountain (Nafusa) and to the south of Kabaw when the Arabs arrived in the region. This western mountain region has since been re-named Nafusa after them (Najem, 2004).

Zenatic dialects.

Tunisian Berber Djerba and Libyan coastal Berber Zuwara have the easternmost part of the Zenatic dialects. This is spoken by the population in Zuwara. It is also spoken in a number of villages in mainland Tunisia, on the Isle of Djerba, and it is quite different from Djebel Nafusa dialect which is spoken in Nafusa mountains. However, both dialects share many characteristics (Kossmann, 2013). Zuwara town is inhabited by the Sanhaja tribe which maintained resistance to the Romans throughout the occupation of Libya before the advent of Islam. Some of Sanhaja Berber groups also lived in West Mountains (Najem, 2004).

Ghadames dialect.

This language is spoken in the historical town on the Libyan Algerian-Tunisian border (Ghadames). It is very different from other Berber dialects, although it shares some features with Berbers living in Nafusa Mountain (Kossmann, 2013)**.** The origin of Ghadames population is always a matter of debate, while the majority of the population considered themselves Arabs migrating from Yemen before Islam they may have acquired their language from the neighbouring populations. Others in the population considered themselves Berber belonging to Liwata and Hiwara tribes (Najem, 2004)**.**

Awdjila dialect.

This dialect is spoken in one of the oasis in the eastern part of Libya (Awdjila), in Djalu region, this dialect has some retention shared with Ghadames dialect: however, it is best considered an entity on its own (Kossmann, 2013). Awdjila inhabitants are a combination of Hiwara and Liwata tribes that live the oasis of Siwa, Awjila, Ghadames and Zuwayla. (Najem, 2004). The Hiwara tribe also live in Tripoli and Misrata (Najem, 2004).

The Libyan-Egyptian Oasis dialect.

This dialect has a number of commonalities with Ghadames and Zenatic dialect spoken in three eastern Saharan oases; two of them in Libya: Sokna in Al-Djufra region and El-Fogaha in Fezzan in addition to Siwa (15,000 people) in western Egypt. (Kossmann, 2013). These Oasis are inhabited by Hiwara tribes that speak the Libyan-Egyptian dialect (Najem, 2004).

Tamasheq dialect.

The Libyan Tuareg live mostly in the west of Libya south of Ghadames, and Ghat. Tamasheq is a block of dialects spoken by Tuareg in Libya, Algeria, Mali, Niger and Burkina Faso. The Ahaggar dialect is spoken by Tuareg living in Ahaggar Mountain between Algeria and Libya while Ghat is the dialect spoken by Tuareg living in Ghat in the southern western part of Libya (Kossmann, 2013).

1.15.5. Libyan Arab

Arab history started 5 kya when the number of tribes such as Saba, Qataban, Main, Qataban, Hiymar were established. The Arab term was initially referred to the nomads who lived in Central and Northern Arabia (Najem, 2004). Arabs arrived in Libya (Cyrenaica) in the east from Arabia through Egypt 642 AD followed by expansion to the south of Libya 643AD. The rest of the Arabs headed to the northwest of Libya in 643AD. The majority of these Arabs were armies belonging to the Yemeni tribes such as the Tujib, Lakhm and Used in addition to al-Muhajrin and al-Ansär, Saudi tribes (Abun-Nasr, 1987). Soon those Arabs were followed by their families to start their permanent settlement in the newly conquered areas in Libya. However, the largest migration of Arabs to North Africa occurred in the eleventh century. These tribes include BanuSulaym and BanuHilal and from Yemen. After that Arabs continued to arrive gradually to the extent that the Amazigh were loosely recognised by others (Najem, 2004).

1.15.6. The Dark-skinned Libyan (Arabized Black)

One of the components of Libya and the North African population is the Arabized Blacks who became part of the Arab culture and language. Most of the Arabized Blacks live in the southern region of Libya, mainly in Fezzan while others live in the cities closer to the Mediterranean coast.

Although the geographical and climatic conditions have prevented sub-Saharan gene flow to Libya in the last 5 kya, the Arab slave trade could have facilitated this migration of Arabized black lineages (Harich et al., 2010). In addition, there was a major migration of a sub-Saharan population that migrated from Africa in 1929 when the slave caravans to came to Fezzan that made up most of the population of Arabized Blacks, some of them then migrated to Tripoli during the Second World War. Another component of the Arabized Blacks is the Tebu, which live on the Libyan border with Chad, Niger and Sudan (Winter and Koch, 1999, Van Waas, 2013b).

1.15.6.1. Tebu

Tebu are a traditionally nomadic Saharan tribe, mentioned by Herodotus. Tebu is a term describing the several closely related tribes that have lived in the south in Niger, Libya and Chad for at least three thousand years (Van Waas, 2013a). Tebu speaks Tebu, a Saharan Latin dialect script. The Tebu population is approximately 120.000 people, split between these countries, with several tens of thousands residing in the south-east of Libya. The Tebu belongs to several clans; however, they are all Muslims and share many cultural values (Van Waas, 2013a).

1.15.6.2. Tuareg

The third components of Arabized Blacks are Tuaregs, who speaks the Berber language, (Van Waas, 2013a). Tuareg are people of nomadic and sedentary life that live in the Sahara and speak Tamazight language the part of Berber language distributed in many countries such as Algeria, Niger. Mali and Burkina Faso, Figure 12 (Sabri, 2011). They have the same identity of the inhabitants of North Africa. They settled in the Sahara region of Africa hundreds of years ago. Ibn Khaldun classified them as a part of "Sanhaja" Berber tribe. Gustave Le Bon the Frenchman said that Berber including Tuareg came from the shores of the Euphrates and the north of Arabia (Sabri, 2011). Furthermore, some historians suggested that Tuareg are descendants of Algaramant who lived in the Fezzan in Libya (Sabri, 2011).

Tuaregs, also live in the southern part of Libya, mainly in the Tibesti Mountains. Several historical reports indicate that the Tuaregs are Arabs or of Semitic origin that migrated to the area and mixed with the local Berber populations (Swei, Van Waas, 2013b).

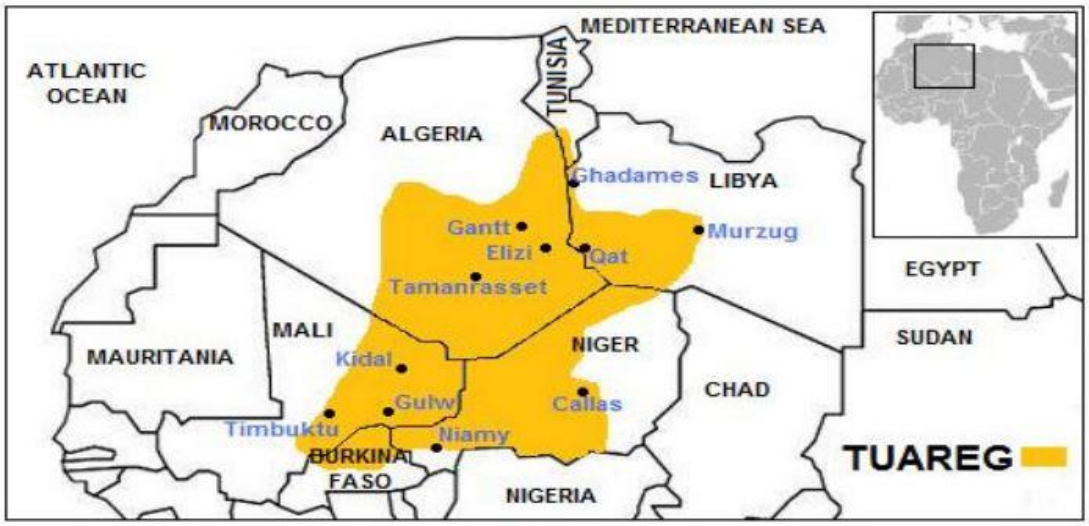

Figure 12. The geographical distribution of the Tuareg population
Taken from (Sabri, 2011)

1.6. Libyan population settlement and industries chronologies

1.16.1. The early Middle Stone Age

The Libyan population settlement grew during the Aterian industry after the end of the settlement of people to Sahara as a result of increasing aridity 60 kya (Garcea and Giraudi, 2006). Comparison between the blade and Taramsa cores of the Tadrart Acacus mountain in south-western Sahara in Libya and Nafusa mountains in north-western Libya Figure 13, revealed that the Aterian period ended in Sahara at least 60 kya, while it continued in the north (Spinapolice and Garcea, 2013).

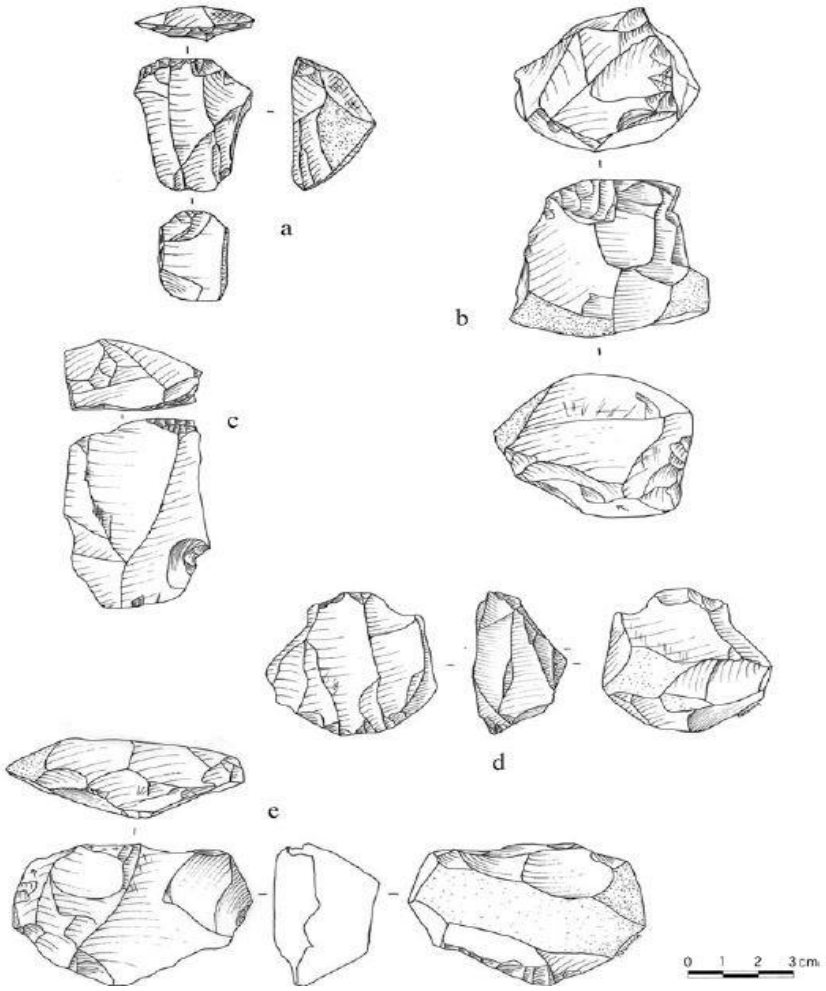

Figure 13. Blade cores and Taramsa cores from Nafusa Mountain
a-c. Blade cores; d-e. Taramsa cores (a, c–d, from SJ-98-28; b, from SJ-00-58; e From SG−00-61). Taken from (Spinapolice and Garcea, 2013)

The first tool in the Early Middle Stone Age were made with the Levalloisian technique. In this age, humans settled in the open-air where there were suitable rock shelters, for instance in the Sahara (Garcea 2001). The Haua Fteah cave is located in Cyrenaica, in the east of Libya, at the foot of the Green Jebel. Stone age tools excavated by McBurney (1967) at the cave (Figure 14) provided one of the most complete Upper Pleistocene and Holocene archaeological sequences (Garcea, 2010).

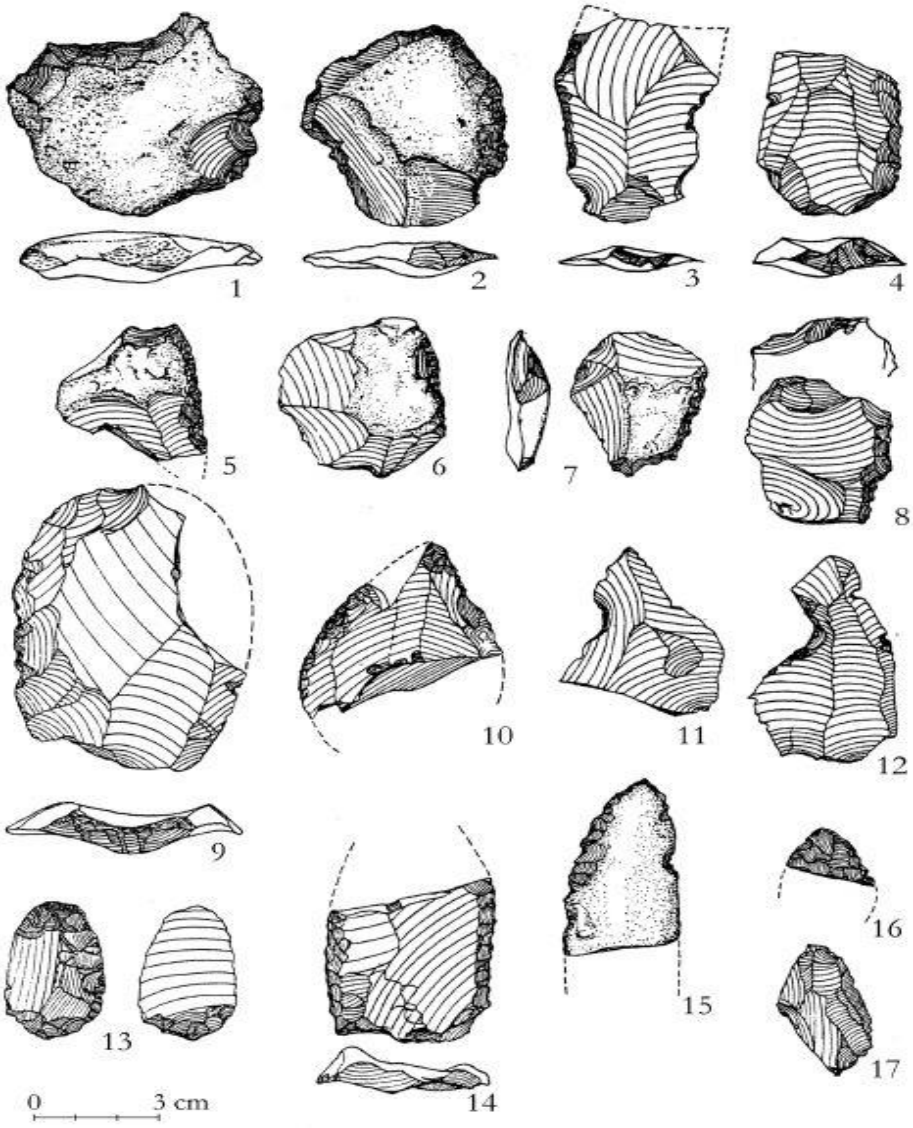

Figure 14. Early Middle Stone Age tools found in Haua Fteah cave

1, 11, 12. Notched flakes; 2–9. Simple side scrapers Taken from(Garcea, 2010)

The Aterian industry is characterized by the tanged stone tools found in the wide geographic area. The Aterian lithic industries from the Jebel Nafusa in Libya reveal proximity with Moroccan and Egyptian Aterian tools, in addition to some from the Levant (Spinapolice and Garcea, 2013). This observation may indicate a possible source of this industry. However, the Aterian has always been thought of as an "African Mousterian" or a "Tanged Mousterian" industry (Spinapolice and Garcea, 2013). Parallel industry in East Libya appeared to be led to producing backed bladelet technologies in Cyrenaica; however, it seems to be more related to the Dabban culture (Spinapolice and Garcea, 2013).

The Haua Fteah cave in north eastern coast was excavated by pioneering Cambridge archaeologist Charles McBurney, who thought that modern humans arrived in the cave at least 40 kya and the cave may contain the key to when and how our species emerged in or colonized North Africa (McBurney et al., 1953, Klein and Scott, 1986). The evidence found includes; two jaws of modern human mandibles (Figure 15) found in the cave that have been dated to 73-65 kya (Douka et al.,2014) (McBurney et al., 1953, Klein and Scott, 1986).

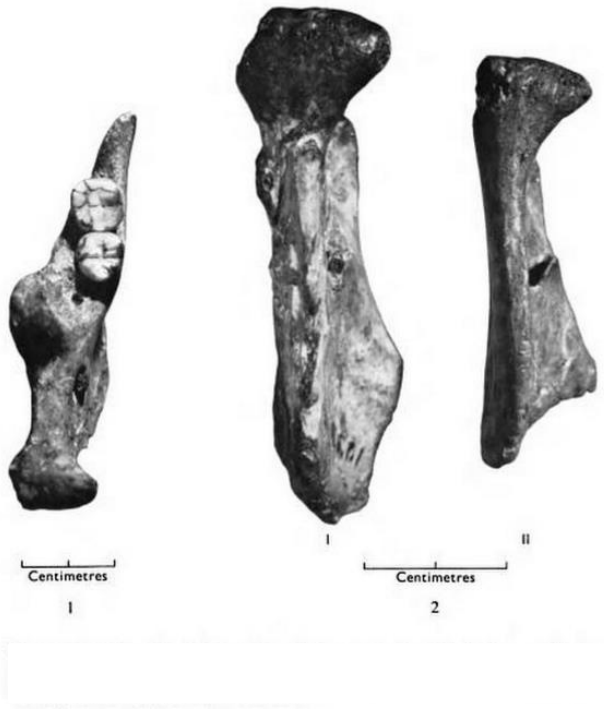

Figure 15. Jaw bones found in Haua Fateh cave

Haua Fteah 1. The cast of the matrix in the mandibular canal, 2. Posterior view of the rami of the Haua Fteah. In each specimen, the lateral part of the condyle is missing (McBurney, 1967)

1.15.7. Iberomaurusian culture in Libya

The Iberomaurusian civilisation started in North Africa 22 kya which is characterised by backed bladelet lithic industry (Figure 16) found throughout North Africa. The Iberomaurusian culture was started by migration of modern humans of Near Eastern origin, carrying mtDNA haplogroup U6, who spread into Cyrenaïca ~40-45 kya and produced the Dabban industry. Several

origins were proposed as a source of this population; Europe, Near East and sub-Saharan Africa. It has also been suggested that this culture evolved *in situ* in North Africa. However, the result of analysis of Ibermauresian remains at Moroccan caves revealed that maternal lineages found in this position were either North African or Eurasian maternal lineages (Kefi et al., 2018).

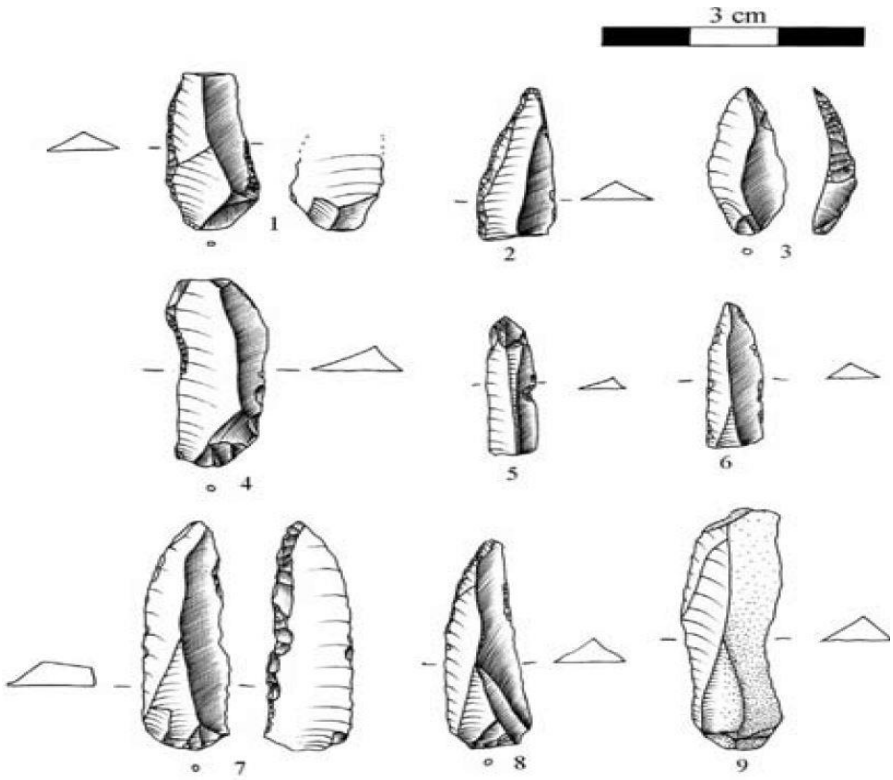

Figure 16. Iberomaurusian lithic tools from Site SJ-00-56 from Nafusa Mountains

Later Stone Age/Iberomaurusian lithic tools from Site SJ-00-56. 1-2-4-6-8: Bladelets with Ouchtata retouch; 3: backed bladelets; 7: bladelet with continuous reversed retouch; 9: bladelet (*drawings by C. Rozera*). Taken from (Barich et al., 2010).

1.15.8. Farmer expansion in Libya

The transition from hunter-gatherers to the farmers was one of the greatest stories in human history. Agriculture started in Fertile Crescent 11 kya and then spread all over the world at different times (Fregel et al., 2018). It has been suggested that a Mesolithic culture Capsin 11 – 7 kya, gave way to the Neolithic transition to agriculture to spread from Near East through 5.5 kya (Fadhlaoui-Zid et

al., 2004). It also has been suggested that that the spread of agriculture in North Africa is associated the arrival of haplogroup J-M304 lineages to North Africa from Arabia and Levant at Neolithic demic diffusion 5.5 kya (Semino et al., 2004). This transition leads to an increase in the diversity of the existing population and ultimately led to the increasing the diversity of ancestors of the indigenous population in North Africa (Fadhlaoui-Zid et al., 2011).

1.15.9. Ancient historical evidence in Libyan Sahara

The Libyan Sahara has been investigated by many European travellers such as Henry Duveyrier, Heinrich Barth, Gustave Nachtegal, Ervin De Bary, Paolo Graziosi and then Fabrizio Mori and the Italian-Libyan Archaeological Mission in Acacus and Messak (Douka et al.,2014).

One of these discoveries was an African black mummy of a three years old child completely wrapped in an envelope of animal skin found in Uan Muhuggiag, in Tadrart acacus in Libya dated to 5.5 kya (Figure 17) indicating human habitation in Libyan Saharan at least since 5 kya (Alsherif, 2014, Mattingly et al., 2001).

Figure 17. Mummy of a child found in Uan Muhuggiag cave

(Assaraya Alhamra Museum, Gallery 4)

Pottery fragments have also been found (red slip ware, fragment of pans, deep casserole jugs, bottles and small and medium amphorae) cover a broad chronological time span, from the end of the 3rd – 2nd century BC to the 6th – 7th century AD in Libya (Figure 18) (Alsherif, 2014, Gatto, 2008, Mattingly et al., 2001).

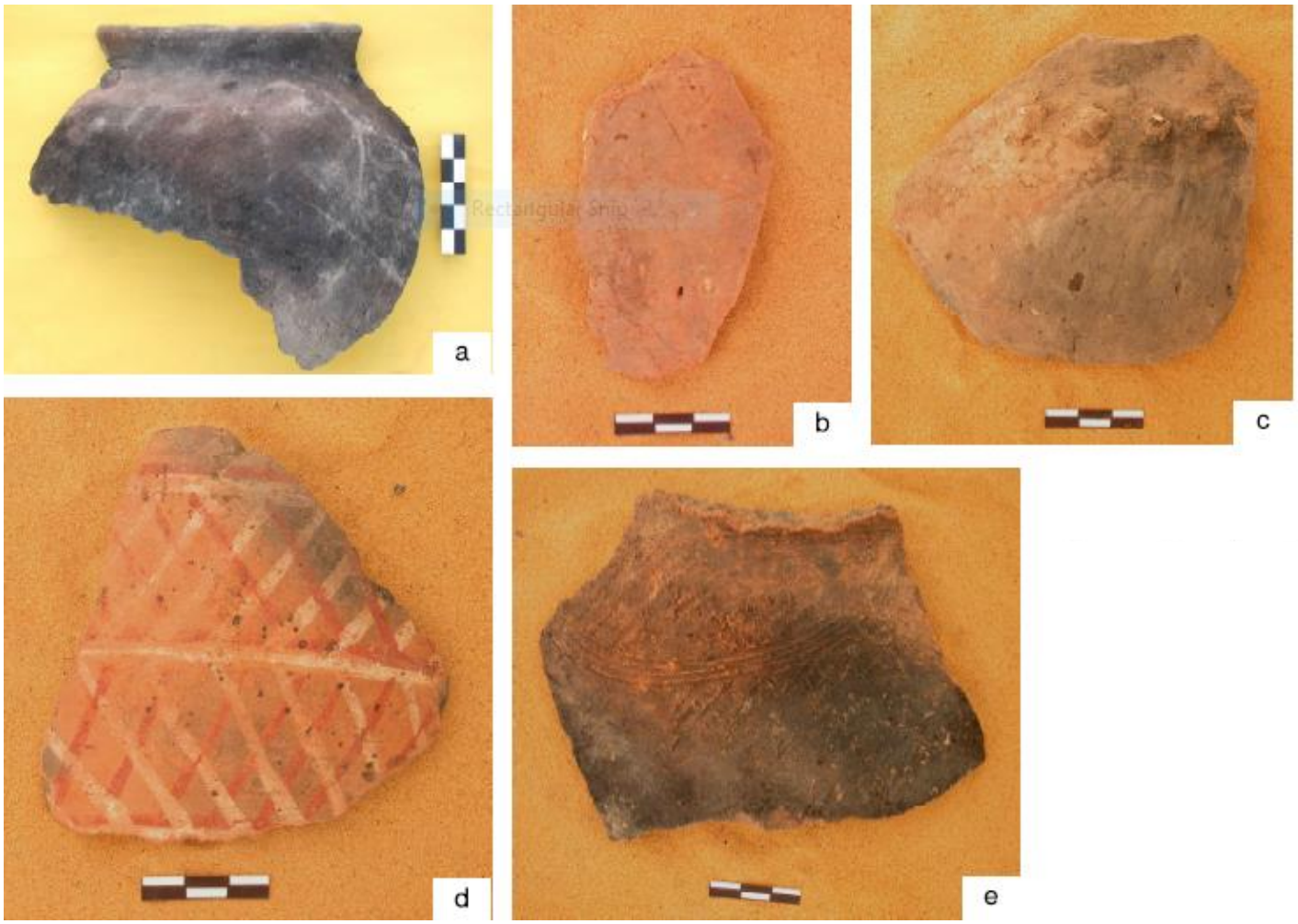

Figure 18. Pottery from Libya (Italian- Libyan mission in Fezzan)

Example of Libyan pottery decoration. a. impressed decoration; b. incised decoration applications; c. Applications; d. painted decoration; e. combined technique: incised and painted (Italian-Libyan Mission in the Acacus and Messak, Sapienza. Università di Roma). Taken from (Maria Gatto, 2010)

Thousands of different styles of caves paintings and rock art dated from 14 kya to 100 ya have been found in Libya (Figure 19) indicating human habitation in the Libyan Sahara (Alsherif, 2014,

Mattingly et al., 2001) and provides a glimpse of human adaptation in the early Holocene of this region (Minozzi et al 2003).

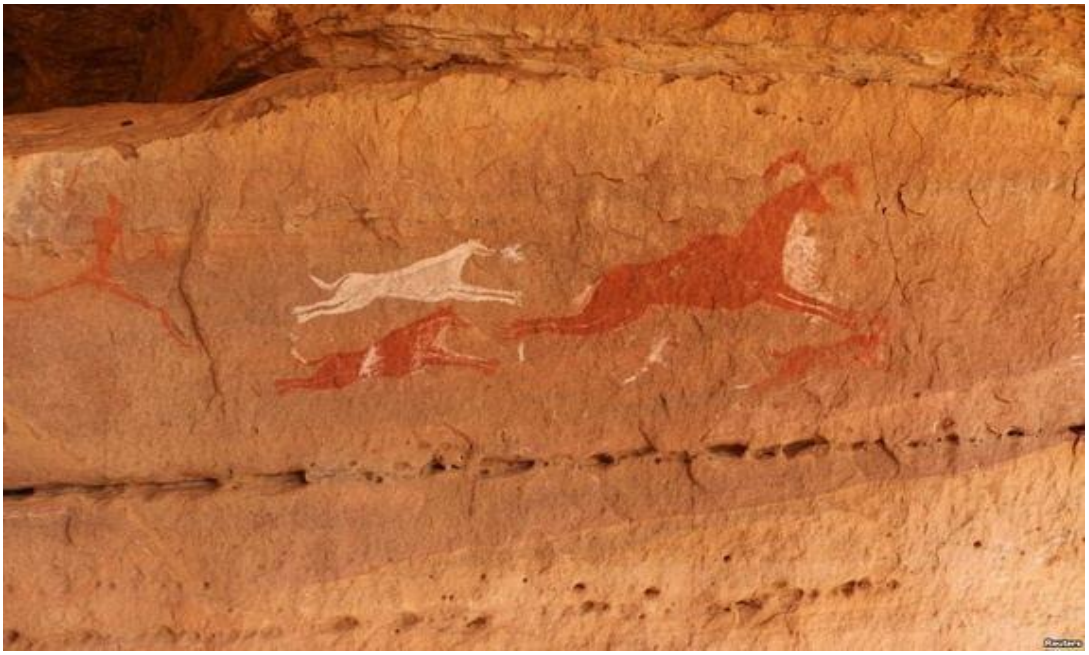

Figure 19. Barbary sheep pursued by hunting dogs in a rock painting of Libyan Sahara

Taken from (Masseti, 2010)

Further evidence of ancient populations have also been uncovered by archaeobotanical evidence in the Fezzan region, wherein the pre-dessert of Tripolitania a Garamantian settlement dated to the first millennium BC, and to the Roman period in Tripolitania (Van der Veen, 1995).

1.15.10. Previous population genetic analysis of the Libyan population

A great deal of genetic research has been done in North Africa populations from Morocco, Algeria, Tunisia and Egypt; however, as a result of the absence of departments in the Libyan universities with interests in the specialism of population or evolutionary genetics, in addition to political restrictions on these types of scientific research, only few studies have been done in these fields on Libyan populations, mainly by Tunisian or Italian researchers (Ottoni et al., 2009, Fadhlaoui-Zid et al., 2011b, Ottoni et al., 2011, Triki-Fendri et al., 2013, Triki-Fendri et al., 2015). Unfortunately, most of these studies were done after 2010 in Tunisia, and the samples were taken from a biased subset of Libyans who were displaced or were receiving treatment outside the country due to the

war in 2010. Also, the majority of these studies were done without discrimination of ethnicity or geographical origin with individuals just being termed Libyan.

The previous main study done by Fadhlaoui et al compared 269 Libyan mtDNA HVSI and HVSII sequences that had no ethnicity recorded with those of other North Africa populations. The study found that West Eurasian lineages were the most predominant in Libyans found at 65%. sub-Saharan L lineages were found at the moderate percentage of 28%, and the indigenous lineages U6 and M1, combined were at 7%. The H haplogroup was the most abundant in this sample being at 17 % (Fadhlaoui-Zid et al., 2011b).

Two Y-chromosome studies done on Libyan subjects by Triki-Fendri showed that the of E-M81 Northwest African haplogroup predominated at (33.7%) followed by Middle Eastern origin haplogroup J(xJ1a, J2)-M304 (27.4%) (Triki-Fendri et al., 2013, Triki-Fendri et al., 2015).

A study performed on the Tuareg population in south Libya using mtDNA and Y-chromosome data have shown that the Tuareg shared a common North African West-Eurasian component with a high percentage of mtDNA H1 lineages were observed, suggesting the genetic drift and recent founder events among Libyan Tuareg (Ottoni et al., 2009). Y chromosome analysis revealed that haplogroup (E-M81, haplogroup E1b1b1b predominated followed by a significant percentage of E-U175, haplogroup E1b1a8 (Ottoni et al., 2011).

1.16. Aims of the project

As described in the introduction, there is a very limited amount of information about mtDNA and Y-chromosome data from distinct Libyan populations. In previous studies, the ethnicity and geographic origins of the majority of the studies done with Libyan populations were not recorded. Those studies that have been conducted involved mostly northern populations mainly from Tripoli (Fadhlaoui-Zid et al., 2013, Fadhlaoui- Zid et al., 2011b, Triki-Fendri et al., 2013, Triki- Fendri et al., 2015) and one on a Touareg ethnic group (Ottoni et al., 2015, Ottoni et al., 2009). In contrast, many Berber Libyan populations, African origin Libyans and Arab Libyans from other locations have not been investigated. Studying these different ethnicities in Libya is mandatory to construct the history of Libya and to address possible population substructure with its impact on match probability estimates.

This study aims to address the dearth in genetic population data from Libya a region that is a corridor of human migration between North Africa and the Middle East and connects Europe with sub-Saharan Africa.

The research examines how males and females have contributed to the Libyan gene pool and investigate if there are any significant genetic similarity or differences between different Libyan populations. mtDNA and Y-STRs analysis will be used in order to shed light on the genetic characters of the studied populations and compare them with other population databases.

The main objectives of this study are to:

1. Examine of mtDNA variation by sequencing the HVS and complete mtDNA genome of Libyan individuals and classify them into specific mtDNA haplogroups to help in determining the maternal population potential source, distribution and settlement history and time.

2. Compare mtDNA variation patterns between the aborigine Berber populations of Libya, the Arabic and African groups, to help resolve the relationship of these populations. Three tiers of assessment will be carried out (i) Geographical comparisons on a global and regional level. (ii) Historical comparisons. (iii) Ethnic comparisons at a local level. This will help in the construction of past relationships between the Libyan population and relationships with their neighbours, including other North African, sub-Saharan and European populations, in addition to Middle Eastern populations. This will also help clarify historical demographic events such as population expansions and migration events.
3. Identify the genetic composition and polymorphism of Y chromosome and distribution of paternally inherited haplogroups via 23 Y-STRs loci analysis in Libyan populations using the PowerPlex® Y23 STR. This will greatly enhance the amount of data available for the Y-STR database of Libya which can be used for phylogenetic and forensic applications purposes and contribute to updating the haplotype information contained in Y-Chromosome STR Haplotype Reference Database (YHRD) and add data on 23 loci haplotypes for the Libyan population, which are not yet available.

Chapter 2

Materials and methods

2. Materials and Methods

2.1. Study population

Buccal swabs were taken from 375 Libyan individuals from different geographical regions in Libya Figure 20, belonging to the three main ethnic backgrounds with ethical consent using swabs. The study population was subdivided into three main ethnic groups of Arabic, Berber and African origin. 175 of these samples were from Berber individuals that included 42 samples from Nafusa Mountain Berbers, 77 coastal Berbers from Zuwara and 56 Saharan Berbers from Ghadames. 168 samples were from Arabs, including 125 Western Libyan Arab, 43 Eastern Libyan Arab. In addition to 32 African origin Libyans. The schematic map below indicates the location of where samples were obtained in this study. A full list of sample codes and locations is given in Appendix Table 18.

As this study uses human biological samples (buccal swabs), informed written consent forms were filled and signed by the volunteers prior to their cheek-cell swabs being obtained. Information obtained from the volunteers including their sex, birth, the birthplace of their parents and their ethnic background are shown in Tables 18 in the appendix. The swabs were then stored at 4 ºC until they were analysed. In this study 375, mtDNA control region sequences were generated, and 199 whole mtDNA genome sequences were generated using the Next-generation sequencing technique Table 17. Also, 219 Y-chromosome microsatellite profiles (76 Arab, 132 Berber and 11 African Libyan) were obtained using 23 chromosome STRs (PowerPlexY-23), Table 19 in the appendix.

Figure 20. Locations of the samples included in this study
Arabic samples in red; Berber sample in purple; African Libyan in green
Taken from https://www.lonelyplanet.com/maps/africa/libya/

2.2. Comparative datasets used

Comparative Libyan datasets used in this study include; mtDNA control region data from 269 Libyan samples (Fadhlaoui-Zid et al., 2011b), 129 samples from the Libyan Tuareg population (Ottoni et al., 2009, Ottoni et al., 2010) and 30 Libyan samples from the work of Badro et al., (2013).

In addition, data were compared with 436 published Y-chromosome STR profiles from; 175 from the Western Libyan population (Triki-Fendri et al., 2015), 47 Libyan Tuareg (Ottoni et al., 2011) and 214 individuals from the Eastern Libyan population (Elmrghni et al., 2012). Also, more than 10000 Y-STR profile from Y-Chromosome STR Haplotype Reference Database (YHRD) were used in PCA and MDS analysis in this study.

More than 4000 published mtDNA sequences were obtained from GenBank and the 1000 Genomes Project (McVean et al., 2012) and other published papers, (Table 1) from Africa, Europe, Near East and Iberian Peninsula, to build up the network of the control region and complete sequence phylogenetic trees in order to calculate date of the sequences and arrival time and make phylogeographic interpretations of the Libyan population. These sequences were also used in statistical analysis and PCA plots generated from this study.

The Iberian population were separated from European in the table 1 due to their genetic influence in North Africa and Libya since the LGM period and the recent spread of Arabs into the Iberia.

Table 1. Worldwide populations used in the comparative analysis

Population	Mitochondrial DNA sequences
Iran	(Derenko et al., 2013)
Kurd	(Richards et al., 2000a), (Quintana-Murci et al., 2004)
Turkey	(Vernesi et al., 2001)
Druze	(Macaulay et al., 1999), (Shlush et al., 2008)
Syria	(Badro et al., 2013), (Vernesi et al., 2001)
Palestine	(Richards et al., 2000a), (Badro et al., 2013)
Lebanon	(Badro et al., 2013, Shlush et al., 2008)
Jordan	(Badro et al., 2013), (González et al., 2008)
Bedouin	(Abu-Amero et al., 2007a)
UAE	(Alshamali et al., 2008)
Kuwait	(Theyab et al., 2012)
Iraq	(Al-Zahery et al., 2003), (Al-Zahery et al., 2011)
Saudi Arabia	(Abu-Amero et al., 2008)
Yemen	(Kivisild et al., 2004), (Cerny et al., 2008)
Sweden	(Pala et al., 2012)
Denmark	(Pala et al., 2012)
Finland	(Hedman et al., 2007)
Italy	(Falchi et al., 2006), (Turchi et al., 2008), (Pala et al., 2012)
Scotland	(Helgason et al., 2001)
England	(Helgason et al., 2001)
Wales	(Pala et al., 2012)
Germany	(Pala et al., 2012), (Tetzlaff et al., 2007), (Brandstätter et al., 2006)
Saami	(Achilli et al., 2005)
Norway	(Pala et al., 2012)
Ashkenazi	(Costa et al., 2013b)
France	(Dubut et al., 2008), (Richard et al., 2008)
Greece	(Irwin et al., 2008)
Slovakia	(Malyarchuk et al., 2008)
Cyprus	(Irwin et al., 2008)
Bulgaria	(Karachanak et al., 2012)
Sardinia	(Francalacci et al., 2013, Olivieri et al., 2017)
Bosnia	(Malyarchuk et al., 2003)
Croatia	(Babalini et al., 2005)
Greece	(Brandstätter et al., 2007)
Ukraine	(Quintana-Murci et al., 2004)
Andalusia	(Hernandez et al., 2014)
Basque	(Cardoso et al., 2011)
Spain	(Barral-Arca et al., 2016)
Portugal	(Barral-Arca et al., 2016)
Egypt	(Saunier et al., 2008), (Coudray et al., 2009)
Canary islands	(Maca-Meyer et al., 2004)
Mauritania	(González et al., 2006)
Algeria	(Bekada et al., 2013), (Macaulay et al., 1999), (Plaza et al., 2003)
Libya	(Fadhlaoui- Zid et al., 2011b), (Badro et al., 2013), (Ottoni et al., 2009)
Morocco	(Turchi et al., 2009), (Plaza et al., 2003),
Tunisia	(Cherni et al., 2009) (GeneBank), (Frigi et al., 2017a), (Fadhlaoui- Zid et al., 2004)
Ethiopia	(Kivisild et al., 2004)
Kenya	(Brandstatter et al., 2004)
Sudan	(Černý et al., 2007), (Afonso et al., 2008)
Burkina-Faso	(Pereira et al., 2010)
Mali	(Pereira et al., 2010), González et al., 2006)
Niger	(Pereira et al., 2010)
Chad	(Cerezo et al., 2011)
Nigeria	(Watson et al., 1997)
South Africa	(Chen et al., 2000, Klein, 2001)
Cameroon	(Coia et al., 2005)
Ghana	(Fendt et al., 2012)

2.3. mtDNA molecular analysis

2.3.1. DNA isolation

Total genomic DNA was isolated from the buccal swab with the QIAamp® DNA Blood kit (QIAGEN®) according to manufactures instructions for buccal swabs as the following.

Procedure

Buffers and protease were prepared according to the QIAGEN® kit instructions.

1. The cotton heads of the swabs were separated from the wooden sticks using a new sterile scalpel for each sample; the cotton was then transferred into a 1.5 ml microfuge tube, and 400 µl of phosphate buffered saline (PBS) was added.
2. 20 µl QIAGEN proteinase K was then added and followed by 400 µl Buffer AL; then the mixture immediately and thoroughly mixed by vortexing for 15 s.
3. The sample was incubated for 30 minutes at 56°C, after which, the sample was centrifuged to remove condensation from the lid.
4. 400 µl of ethanol (96–100% v/v) was then was added to the sample and vortexing. After this, the sample was centrifuged to remove drops from inside the lid.
5. 700 µl of the solution from the previous step was then carefully applied to a QIAamp Mini spin column in the collection tube, and then the cap was closed. The sample was centrifuged at 8000 rpm for 1 min. The column was then placed in a clean 2 ml collection tube, and the tube containing the previous filtrate was discarded.
6. After that, the previous step was repeated by applying up to 700 µl of the remaining mixture from step 4 to the QIAamp Mini spin column.
7. The QIAamp Mini spin column was then opened, and 500 µl Buffer AW1 was added without wetting the rim, the tube was then closed and centrifuged at 8000 rpm for 1 min. The

QIAamp Mini spin column was then placed in a clean 2 ml collection tube, and the tube containing the filtrate was discarded.

8. The QIAamp Mini spin column was then carefully opened, and 500 µl Buffer AW2 was added without wetting the rim. The tube was then closed and centrifuged at 14,000 rpm for 3 min.
9. The column was then placed in a new 2 ml collection tube, and the old collection tube with the filtrate was discarded and then centrifuged at full speed for 1 min.
10. The QIAamp Mini spin column was then placed in a clean 1.5 ml microcentrifuge tube, and the collection tube containing the filtrate then discarded.
11. Finally, the QIAamp Mini spin column was carefully opened and 150 µl Buffer AE added and the sample incubated at room temperature (15–25°C) for 1 min. This was then centrifuged at 8000 rpm for 1 min. The eluate sample containing the genomic DNA was then stored at –20°C.

2.3.2. PCR amplification of mtDNA control region

The mitochondrial HVSI and II regions (Figure 21) were amplified for all samples (Table 2). In addition, the mtDNA of the entire mtDNA control region was amplified for some samples using 2 pairs of primers.

L1599 F 5'-CTCCACCATTAGCACCCAAAGC-3'(22 bp) H**408R** 5'-TGTTAAAAGTGCATACCGCCA-3'(21 bp)

16413F 5'-TGAAATCAATATCCCGCACA-3'(20 bp) **739R** 5'-GTGGTGATTTAGAGGGTGAA-3'(20 bp)

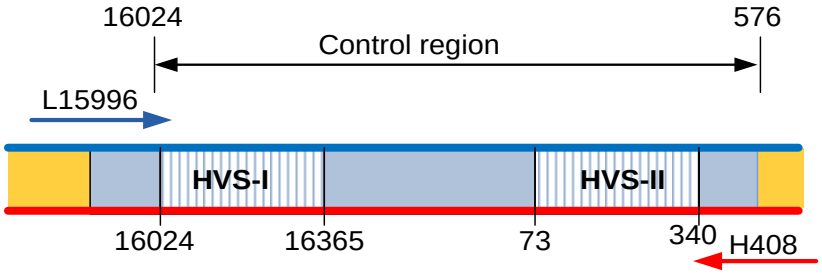

Figure 21. The position of primers used to amplify the mtDNA control region

Table 2. PCR reaction master mix

Reagents	Volum per reaction (µl)
Enhancer solution P (Green buffer) 5X (peQlab)	**5 µl**
Reaction buffer Y (Red buffer) 10X (peQlab)	**2.5 µl**
Forward primer (10pmol/µl)	**0.75 µl**
Reverse primer (10pmol/µl)	**0.75 µl**
peqGOLD dNTP-mix 10mM each (peQlab)	**0.5 µl**
Taq-DNA-Polymerase 5u/µl (peQlab).	**0.2 µl**
Total volume	**9.7 µl**

The annealing temperature (T_a^{OPT}) of the primers was optimised using a C1000Tm Thermal Cycler Bio-Rad Inc. This was achieved using gradient thermocycler with a range of different temperatures above and below the calculated T_a, Table 3.

Table 3. PCR cycling protocol

No	Step	Temperature	Time	Number of Cycles
1	Initial Denaturation	95°C	2 minutes	1 Cycles
2	Denaturation	95°C	30 seconds	35 Cycles
	Annealing	56°C	30 seconds	
	Extension	72°C	70 seconds	
3	Final Extension	72°C	5 minutes	1 Cycles
4	Soak	4°C	Indefinite	1 Cycles

As some of the haplogroups cannot be assigned using just the control region, several samples were sequenced for a different position in the mtDNA genome using different sets of primers (Table 4).

Table 4. Primers used for PCR amplification and sequencing of mtDNA
F; forward primer, R; reverse primer

PCR	Amplification Primers	Sequencing	Sequencing Primers	Samples
L1599 F	5'-CTCCACCATTAGCACCCAAAGC-3'(22 bp)	**L15996F**	5'-CTCCACCATTAGCACCCAAAGC-3' (22 bp)	**All**
H**408R**	5'-TGTTAAAAGTGCATACCGCCA-3'(21 bp)	H**408R**	5'-TGTTAAAAGTGCATACCGCCA-3' (21 bp)	
16413F	5'-TGAAATCAATATCCCGCACA-3'(20 bp)	**16413F**	5'-TGAAATCAATATCCCGCACA-3'(20 bp)	**J1c1**
739R	5'-GTGGTGATTTAGAGGGTGAA-3'(20 bp)	**739R**	5'-GTGGTGATTTAGAGGGTGAA-3'(20 bp)	
		131R	5'-ACAGATACTGCGACATAGGG-3'(20 bp)	**Poly c**
2007F	5'-TGGTGATAGCTGGTTGTCCA-3'(20 bp)	**2007F**	5'-TGGTGATAGCTGGTTGTCCA-3'(20 bp)	**R, HV, H**
3169R	5'-GGAAGGCGCTTTGTGAAGTA -3'(20 bp)	**3169R**	5'-GGAAGGCGCTTTGTGAAGTA -3'(20 bp)	**R, HV, H**
2835F	5'-ATCCAATAACTTGACCAACG-3'(20 bp)	**2835F**	5'-ATCCAATAACTTGACCAACG-3'(20 bp)	**J1c1**
3836R	5'-TAGCCGTTTACTCAATCCTC-3'(20 bp)	**3836R**	5'-TAGCCGTTTACTCAATCCTC-3'(20 bp)	
5162F	5'-TCGCACCTGAAACAAGCTAA-3'(20 bp)	**5162F**	5'-TCGCACCTGAAACAAGCTAA-3'(20 bp)	
6096R	5'-TTACAAATGCATGGGCTGTG-3'(20 bp)	**6096R**	5'-TTACAAATGCATGGGCTGTG-3'(20 bp)	
6643F	5'-CCTACCAGGCTTCGGAATA-3'(20 bp)	**6643F**	5'-CCTACCAGGCTTCGGAATA-3'(20 bp)	
7818R	5'-AGGGCGATGAGGACTAGGAT-3'(20 bp)	**7818R**	5'-AGGGCGATGAGGACTAGGAT-3'(20 bp)	
9183F	5'-CCTCTACCTGCACGACAACA-3'(20 bp)	**918F**	5'-CCTCTACCTGCACGACAACA-3'(20 bp)	
10175R	5'-GCACTCGTAAGGGGTGGAT-3'(19 bp)			
10609F	5'-TAACCCTCAACACCCACTCC-3'(20 bp)	**10609F**	5'-TAACCCTCAACACCCACTCC-3'(20 bp)	
11676R	5'-GCGTTCGTAGTTTGAGTTTGC-3'(21 bp)	**11676R**	5'-GCGTTCGTAGTTTGAGTTTGC-3'(21 bp)	
12913F	5'-TCCAACTCATGAGACCCACA-3'(20 bp)	**12913F**	5'-TCCAACTCATGAGACCCACA-3'(20 bp)	
14068R	5'-AGGTGATGATGGAGGTGGAG-3'(20 bp)	**14068R**	5'-AGGTGATGATGGAGGTGGAG-3'(20 bp)	
13714F	5'-GGAAGCCTATTCGCAGGATT-3'(20 bp)	**13714F**	5'-GGAAGCCTATTCGCAGGATT-3'(20 bp)	**J1c1**
14856R	5'-AGGAGTGAGCCGAAGTTTCA-3'(20 bp)	**14856R**	5'-AGGAGTGAGCCGAAGTTTCA-3'(20 bp)	**J1c1, HV**
15195F	5'-TATCCGCCATCCCATACATT-3'(20 bp)	**15195F**	5'-TATCCGCCATCCCATACATT-3'(20 bp)	
16401R	5'-TGATTTCACGGAGGATGGTG-3'(20 bp)	**16401R**	5'-TGATTTCACGGAGGATGGTG-3'(20 bp)	

2.3.3. PCR amplification of whole mitochondrial genome

To allow sequencing of the entire mtDNA genome. 12 samples were amplified by divided the sample into 18 overlapping PCR fragments of approximately 900 base pairs bp each; using a set of 18 primer pairs with matching annealing temperatures (Table 4).

PCR reaction master mix, reaction condition of implication and cycling protocol are identical to ones applied for control region mentioned above.

The PCR reaction mix was prepared as in Table 2. After that, a master mix was thoroughly mixed, and 9.7 µl of the mixture was dispensed into 0.2 ml PCR tubes. 13.3 µl nuclease-free water then added. Finally, 2 µl of template DNA (50 ng) or water as a negative control was added to form 25 µl. The samples were then briefly centrifuged and placed in a thermal cycler.

2.3.4. PCR product detection

Visualization of PCR products was achieved by electrophoresis PCR productivity on an agarose gel As follow: 1**.** 0.5 g of agarose (Flowgen) powder was dissolved in 50 ml of 1X TBE buffer in a conical flask to prepare 1% w/v agarose gel

2**.** The agarose was then heated in a microwave oven to melt it completely, and 3 of µl peqGreen dye (Peq labs) was added. While still hot (50 C°) the solution was then poured into a casting tray in the electrophoresis tank with combs in place and left for 30 minutes to solidify. Once the agarose had set, 1×TBE was then added to the electrophoresis tank to submerge the gel.

3. To determine the success of the PCR and estimate the quantity and size of the PCR products; 5 µl of the DNA sample or 7 µl of peqGOLD DNA-Ladder Mix (0.5 mg DNA/ml) (peQlab) was mixed with 1 µl of blue/orange 6X loading dye (Promega) and injected into the wells using a micropipette. The samples were then electrophoresed at 100 V for 20 minutes.

4**.** After 20 min, the PCR products were visualised on a UV transilluminator and photographed using a UVP imaging system.

5. The size and quantity of PCR product was estimated by comparing the intensity of the sample bands to the reference DNA ladder

2.3.4. PCR product purification

PCR products were purified using the QIAquick PCR purification kit as follows.

1. PB Buffer was added to the PCR product at a 5 to 1 ratio (100 µl PB Buffer: 20 µl PCR products) and the solution, was centrifuged at 13000 rpm for 1 min.

2. The QIAquick column was then placed in a 2 ml collection tube, and the DNA added to the column and the solution centrifuged at 13000 rpm for 1 min, and the filtrate discarded.
3. After that, the QIAquick column then placed in a clean 2ml collection tube, and 750 µl PE buffer was added to the column. The solution then centrifuged at 13000 rpm for 1 min, and the filtrate was discarded.

4. The QIAquick column was then placed in a clean 2ml collection tube and centrifuged again at 13000 rpm for 1 min, and the filtrates were discarded.
5. Finally, The QIA quick column was placed in a clean 1.5 ml centrifuge tube and 50 µl EP buffer added to the centre of the QIA quick column. This was then centrifuged at 13000 rpm for 1 min, and the filtrate was containing the purified PCR product was collected.

2.3.5. PCR fragment sequencing

For Sanger DNA sequencing 15 µl of the purified PCR products contain 10 ng DNA/µl was added to 2 µl of a single primer (10pmol/µl), (Table 5) in a separate labelled and barcoded 1.5 ml tube. The samples were then sent to sequence at Eurofins Genetic Services Limited for sequencing on an ABI 3730xl genetic analyser.

2.3.6. PCR amplification of mtDNA for Next Generation Sequencing

As well as using traditional PCR and Sanger sequencing, the whole mitochondrial genome was also amplified using Next Generation Sequencing for 187 targeted samples selected base on control-region variation, in two overlapping PCR fragments of around approximately 9000 bp each Table 5. The PCR reaction master mix was prepared as in Table 6, and 27 µl of the mixture was dispensed into PCR tubes. 22 µl nuclease-free water then added. Finally, 1 µl template DNA (0.1-0.5 µg) or water as a negative control was added to form 50 µl. The samples were then briefly centrifuged and placed in a thermal cycler. Optimised PCR conditions are given in table 7.

Table 5. 4 pairs of nested primers used for PCR amplification and sequencing primers

PCR	Amplification Primers	Sequencing	Sequencing Primers	Samples
5871F	5'- GCTTCACTCAGCCATTTTACCT-3'(22 bp)	**5871F**	5'- GCTTCACTCAGCCATTTTACCT-3'(22 bp)	**All**
13829R	5'- AGTCCTAGGAAAGTGACAGCGA -3'(22 bp)	**13829R**	5'- AGTCCTAGGAAAGTGACAGCGA -3'(22 bp)	
13477F	5'-GCAGGAATACCTTTCCTCACAG-3'(22bp)	**13477F**	5'-GCAGGAATACCTTTCCTCACAG-3'(22 bp)	**All**
6345 R	5'-AGATGGTTAGGTCTACGGAGGC-3'(22bp)	**6345 R**	5'-AGATGGTTAGGTCTACGGAGGC-3'(22bp)	

Table 6. PCR reaction setup

Reagents	Vol per Reaction (µl)
Nuclease-Free Water	**21 µl**
GoTaq® Long PCR Master Mix (PROMEGA) 2X	**25 µl**
Forward primer (10pmol/µl)	**1 µl**
Reverse primer (10pmol/µl)	**1 µl**
DNA template	**2 µl**
Total volume	**50 µl**

Table 7. PCR cycling protocol

No	Step	Temperature	Time	Number of Cycles
1	Initial Denaturation	94°C	2 minutes	1 Cycles
2	Denaturation	94°C	30 seconds	30 Cycles
	Annealing	55°C	30 seconds	
	Extension	65°C	9 minutes	
3	Final Extension	72°C	10 minutes	1 Cycles
4	Soak	4°C	Indefinite	1 Cycles

2.3.7. **Purification of long PCR products.**

Before the start, the membrane was washed by add 1-5 volumes of 95% v/v ethanol as follows:

1. 40 µl of membrane binding solution was added to 40 µl of the 10 ng PCR amplification products and pipetted onto the SV Minicolumn (Promega ®) in a collection tube.

2. This was incubated for 1 minute at room temperature.

3. The SV minicolumn assembly was then centrifuged the in a microcentrifuge at 16,000 × *g* (14,000rpm) for 1 minute.

4. The SV Minicolumn was then removed, and the liquid in the collection tube was discarded, and the SV Minicolumn returned to the collection tube.

5. 700 µl of membrane wash solution was then added and the SV minicolumn assembly centrifuged for 1 minute at 16,000 × g (14,000rpm).

6. The liquid in the collection tube was then discarded and the SV Minicolumn returned to the collection tube.

7. 500µl of membrane wash solution was then added and SV Minicolumn assembly centrifuged the for 5 minutes at 14,000 rpm.

8. The liquid in the collection tube was then discarded, and the SV Minicolumn returned to the collection tube and the column assembly recentrifuged for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.

9. The SV minicolumn was then carefully transferred to a clean 1.5ml microcentrifuge tube, and 50µl of nuclease-free water was then directly applied to the centre of the column.

10. Finally, the microcentrifuge tube was incubated at room temperature for 1 minute and centrifuged for 1 minute at 14,000 rpm and the eluate containing the DNA stored at 4°C or –20°C.

2.3.8. DNA quantitation and normalization of PCR products for NGS.

This reason for this step was to normalise the quantity of the two long PCR fragments on. The two PCR products were quantified using Qubit® Fluorometer. https://www.thermofisher.com/uk/en/home/industrial/spectroscopy-elemental-isotope-analysis/molecular-spectroscopy/fluorometers/qubit/qubit-fluorometer.html

Procedure

Qubit® Fluorometer was calibrated using a calibration solution as per manufacture instructions. https://support.illumina.com/content/dam/illuminasupport/documents/documentation/chemistry_documentation/samplepreps_nextera/nextera-xt/nextera-xt-library-prep-reference-guide-15031942-03.pdf

1. A Qubit® working solution was prepared by adding 1µL of Qubit® reagent to 199 µL of Qubit® buffer solution to form 200 µL in each of the tubes.
2. 1µL of the long PCR product sample was then added to the tube and mixed by vortexing 2–3 seconds.
3. The tubes were then incubated at room temperature for 2 minutes.

After normalisation equal volume of the two fragments, the samples proceeded to Nextera® XT DNA library preparation.

2.3.9. NGS sequencing of the whole mitochondrial genome using Illumina

The basic concept of next-generation sequencing (NGS) technology is that DNA polymerase enzyme catalyses the incorporation of fluorescently labelled deoxyribonucleotide triphosphates (dNTPs) into a DNA template in cycles of DNA synthesis. After that, fluorophore excitation identifies the nucleotides at the point of incorporation during each cycle. The main advantage of this technology over capillary electrophoresis sequencing (CE) is that NGS does this process across millions of fragments at the same time. (Bentley et al., 2008). Moreover, compared to the Sanger method, NGS had a massive advantage in term of throughput and cost were using its HiSeq X Ten sequencing system it can produce 1.6-1.8 Tb high-quality data within three days Figure 22.

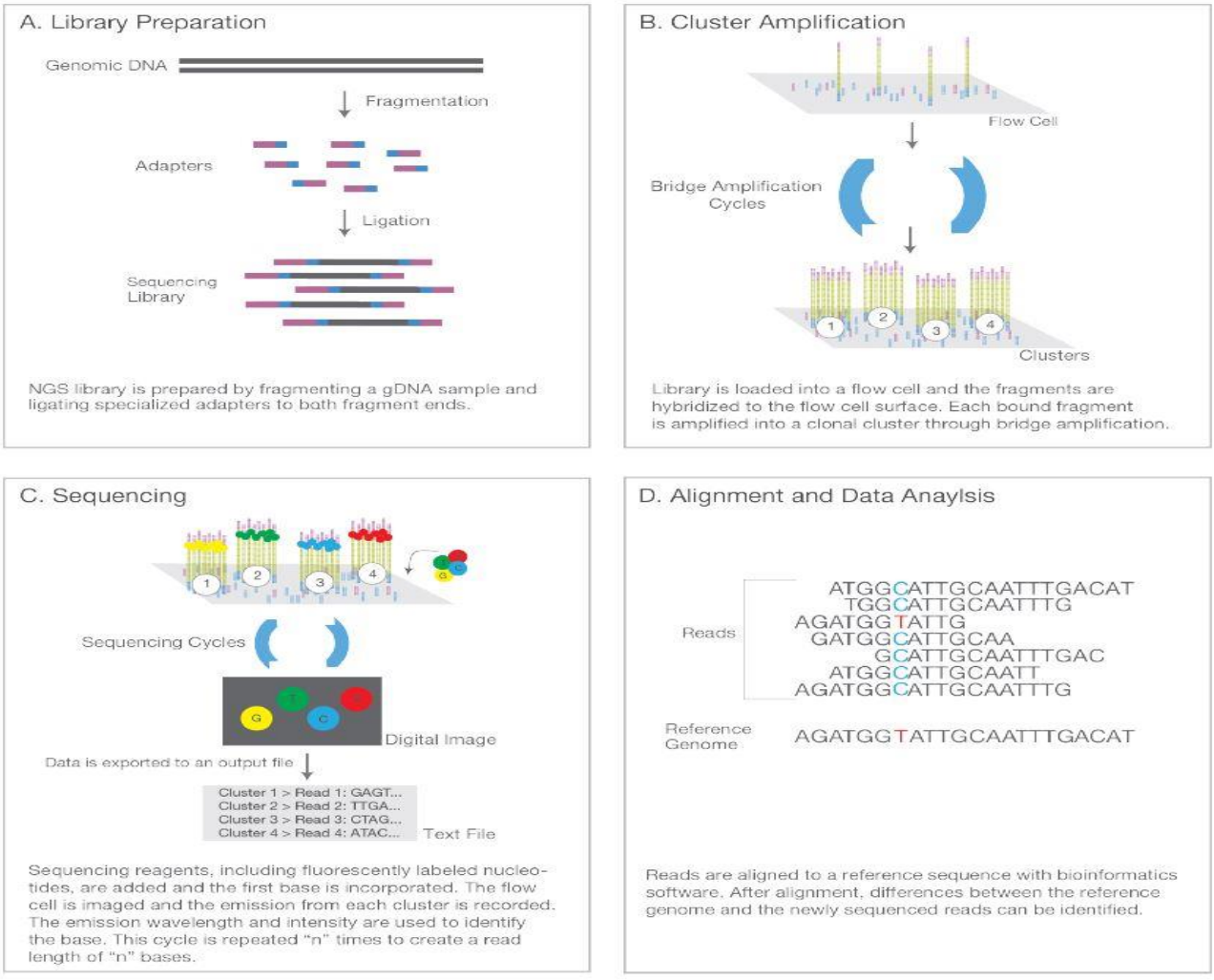

Figure 22. The four steps of NGS sequencing

(A) Library preparation (B) Cluster generation (C) Sequencing and (D) Data analysis.
Taken from I. Illumina 2015 [online] Available: http://www.illumina

2.3.10. Nextera® XT DNA library preparation

To prepare the library, the template DNA was fragmented using an enzymatic DNA fragmentation, and adapter sequences were added to each DNA template to perform multiplexed sequencing libraries within a one tube Nextera XT tagmentation reaction.

2.3.10.1. Tagmentation of long PCR products

In this step, the DNA sample was tagmented using Nextera transposome, and the adapters were tagged to each tagment in a single step that paved the way for to amplification by PCR.

Procedure

10 µl of tagment DNA buffer (TD) was added to each plate well followed by adding 5 µl of normalised DNA (1ng/µl), then 5 µl of Amplicon tagment mix (ATM) was added to each well and mixed gently. After that, all samples centrifuged at 1500 rpm at 20°C for 1 minute.

Finally, the samples were placed in a pre-programmed thermal cycler at 55°C for 5 minutes then held at 10°C.

To neutralise the samples, 5µl of neutralising tagment buffer (NT) was added immediately to each well and mixed gently; then all samples centrifuged at 1500 rpm at 20°C for 1 minute. The PCR plate containing 25 µl tagmented and neutralised DNA was then incubated at room temperature for 5 minutes.

2.3.10.2. Amplification of Libraries

In this step tagmented DNA was amplified using a limited-cycle PCR program (Table 8). The amplification step includes adding the Index 1 (i7), Index 2 (i5) (complementary to the ones in the flow cell), and index adapter sequences (needed for cluster formation) to the tagmented DNA from the last step. Firstly, adaptor Index 1 (i7), hybridise with the complementary oligo in the flow cell in the MiSeq followed by forming a bridge by hybridisation between the Index 2 (i5) and its complementary in the flow cell.

Procedure

15 µl of Nextera PCR Master Mix (NPM) was added directly to the 25 µl of tagmented DNA from the previous step. Then 5 µl of index primer two was added to each column and 5 µl of index primer one were added to each row. The plate then centrifuged at 1500 rpm at 20°C for 1 minute. Finally, the samples were amplified on a thermocycler programmed as shown in Table 8.

Table 8. PCR cycling protocol for NGS amplification

Temperature	Time	Number of Cycles
72°C	3 minutes	1 Cycles
95°C	30 seconds	1 Cycles
95°C	10 seconds	12 Cycles
55°C	30 seconds	
72°C	30 seconds	
72°C	5 minutes	1 Cycles
10°C	Indefinite	

2.3.10.3. Procedure for clean up libraries

This step performed to purify the library DNA and remove short library fragments using AMPure XP beads.

Procedure

The plate was centrifuged at 1500 rpm at 20°C for 1min, then 50 µl of PCR products from each well of the PCR plate transferred to corresponding wells of a new plate. After that, the beads were vortexed for 30 sec, and 90 µl of AMPure XP beads added to each well and mixed gently. The plate was then incubated at room temperature for 5 min. In the following step, the plate was put on a magnetic stand for 2 minutes, and the supernatant was discarded carefully. After that 200 µl of freshly prepared 80% v/v ethanol was added and the plate incubated at room temperature for 30 sec.

The supernatant was then carefully discarded. Again 200 µl of freshly prepared 80% v/v ethanol was added, and the plate incubated at room temperature for 30 sec and the supernatant was carefully discarded. After 15 min the plate was removed from the magnetic stand, and 52.5 µl of resuspension buffer (RSB) was added and centrifuged at 1800 rpm for 2 minutes. After that, the samples were incubated at room temperature for 2 minutes.

Then samples were then placed on a magnetic stand for ~2 minutes. Finally, 50 µl of supernatant was transferred to a new hard-shell 96 well PCR plate.

2.3.10.4. Normalizing Libraries

The reason for this step was to normalise the libraries to ensure equal library representation. This was done by normalizing indexed libraries to 2 nM in the Nextera dilution plate using Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20. After normalisation, an equal volume of the normalised library was aliquoted and diluted in hybridisation buffer. Finally, the samples were denatured by heating and then loaded in the MiSeq flow cell.

2.4. Y-STRs analysis

Prior to the purified genomic DNA was quantified using Qubit fluorometer and the normalised as in (2.3.8).

2.4.1. PCR Amplification

PowerPlex® Y23 5X master mix and PowerPlex® Y23 10X primer pair mix from Promega were briefly centrifuged then vortexed for 15 seconds, then the final volume of each reagent listed in Table 9, was added to a clean 0.2ml tube. Reaction mixes were then mixed and dispensed into PCR tubes. The template DNA and nuclease-free water were then added to the reaction mix, and positive and negative controls were prepared.

Table 9. PCR reaction setup for PowerPlex Y-23 STR amplification

Reagents	Vol per Reaction (µl)
PowerPlex® Y23 5X Master Mix	**5.0µl**
PowerPlex® Y23 10X Primer Pair Mix	**2.5 µl**
template DNA (0.5ng)	up to **17.5µl**
Nuclease-free water	**Up to 25 µl**

Following preparation of the full reaction mix, samples were amplified on a thermocycler programmed as shown in Table 10.

Table 10. PCR cycling protocol

No	Step	Temperature	Time	Number of Cycles
1	(hot-start to activate DNA polymerase)	96°C	2 min	1 Cycles
2	Denaturation	94°C	10 sec	30 Cycles
	Annealing	61°C	1 min	
	Extension	72°C	30 sec	
3	Final Extension	60°C	20 minutes	1 Cycles
4	Soak	4°C	Indefinite	

2.4.4. Capillary Electrophoresis (Genetic Analyser).

Loading buffer was prepared by mixing 9.5 µl WEN ILS 500 Y23 (Promega) and 0.5 µl Hi-Di Formamide. After that the loading buffer was vortexed for 10–15 seconds to mix. 10 µl of the

mixture was then added to each well, and 1µl of the amplified sample (or 1µl of PowerPlex® Y23 Allelic Ladder Mix) were added to each well. The samples were then denatured for 3 min at 95°C; then cooled in the thermal cycler 4°C. The samples were then loaded on the tray. All samples including the standards were analysed using a ABI PRISM 3130/3130xl genetic analyser.

2.5.Data Analysis

2.5.1. Mitochondrial DNA data acquisition

All of the 375 samples from Libyan individuals from three ethnic backgrounds were amplified and sequenced for the entire control region using forward and reverse primers that cover the HVS-I region from 16024 to 16365 and the HVS-II region from 73 to 340. In order to classify some samples further as well as these regions, PCR was also performed to clone the rest control region up to nucleotide 700 of the mitochondrial genome. After the first HVS classification, several fragments in the mtDNA coding region were sequenced for a particular location in the coding region in order to refine further and confirm some haplogroups, and 12 individuals had the entire mitochondrial genome PCR amplification and Sanger sequenced.

Sanger sequence data in FASTA and SCF formats for the PCR products were generated using an ABI 3730xl DNA analyser. In addition, 201 sequences representing the different lineages found among Libyan population were selected for whole genome sequencing using next-generation sequencing technique. The data were outputted from MiSeq flow cell in paired-end FASTQ format.

2.5.2. Data manipulation and variants scoring

All of the resultant Sanger sequences electrophoretograms were aligned and scrutinised against the rCRS (GenBank ID 251831106) (Andrews et al., 1999) using the Sequencher 5.4 software, http://www.genecodes.com **an**d were carefully checked side by side to assess the quality. (Figure 23). FASTQ sequences that resulted from complete mtDNA genome next-generation sequencing were uploaded to the mtDNA-server https://mtdna-server.uibk.ac.at/index.html for

analysis. FASTQ files were also aligned and scrutinised against the revised Cambridge Reference Sequence (rCRS) with Geneious 6.1.8 software and checked for coverage and data was then collected in FASTA formats that was used in further analysis.

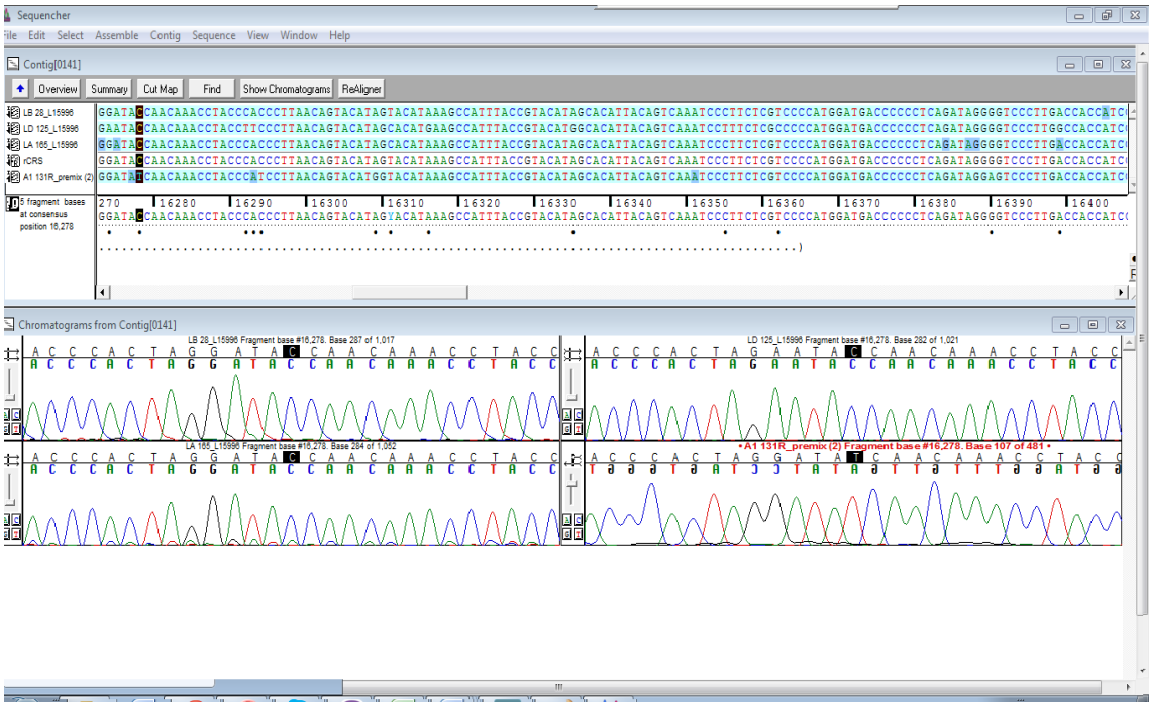

Figure 23. Example of Sequencher 5.4 electrophoretogram interface

2.5.3. Sequencher 5.4

Sequencher is a DNA sequence analysis software that can view and analyse results for Sanger, Next-Generation Sequencing (NGS), and RNA-Seq sequence data. It allows for the alignment of different length nucleotide sequences against the rCRS. Data sequences can be imported into several types of format including Fasta, Nexus, SCF, Genebank and phylip. Any poor quality sequence or with an ambiguous reading was re-sequenced using another primer, usually a reverse primer. Variants were recorded when the aligned positions were different from the rCRS Figure 23. Samples with C-stretch or poly-cytosine (poly-C) phenomenon, after position 16189 in the HVS-I Figure 24, were re-sequenced using reverse primer H 131R (Table 4). In this case, the T nucleotide is situated between two groups of C's, and when a transition polymorphism occurs changing the T to a C, the genetic analyser sometimes fails to read through the lengthened "C-stretch". Because of

different length heteroplasmy between the poly-C and the sequence after the poly-C DNA, the polymerase stutters when this point is reached. As a result, the data beyond the C-stretch is of no interpretable value (Figure 24), (Bendall and Sykes, 1995, Parson et al., 1998, Tetzlaff et al., 2007).

The heteroplasmy per sample was also checked with Geneious software added manually to the Fasta files. mtDNA-server and Mitotool software were also used to obtain a Table of variants in a Microsoft Excel sheet which also contained the information of the samples and identified haplogroups. Positions found different from the (rCRS) were recorded. These nucleotide changes include; transitions when one of the pyrimidine (T or C) is substituted for the other pyrimidine or a purine base is substituted for the other purine (A or G) at a specific position. For instance of C at np 16188 substituted by T (C16188T). Transversions occur when purine substituted for a pyrimidine or vice versa such as T replaced by A at np 13777 (13777a) (Sobrino et al., 2005, Frazer et al., 2007, Consortium, 2010). The other types of DNA modifications included insertions or deletions.

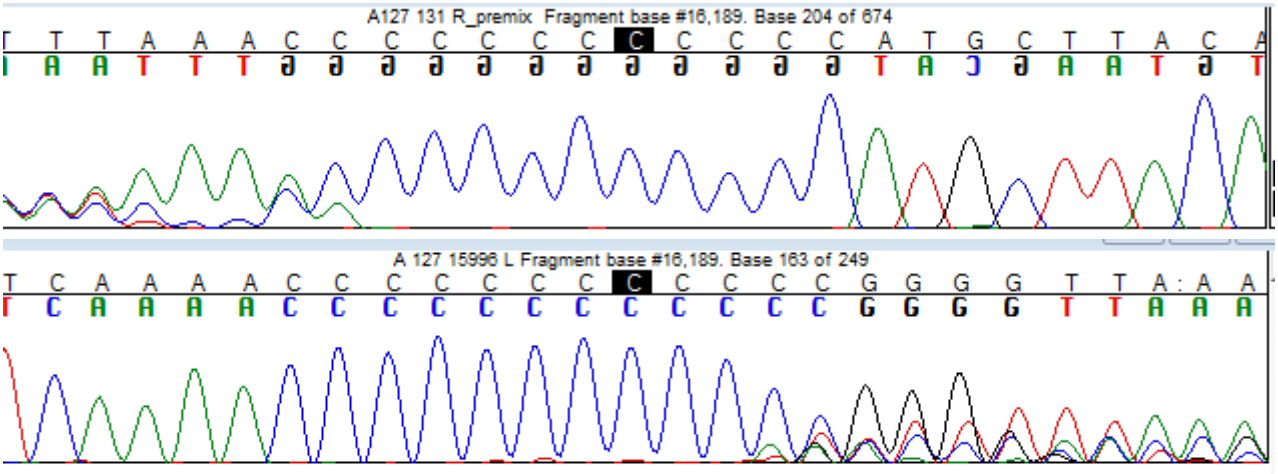

Figure 24. Electrophoretogram showing poly C problem at nucleotide 16189
The transition T>C at 16189 which leads to distortion of the sequence after this point. The sequence A127 was corrected using an H131R primer

2.5.4. Geneious® software 6.0.6

The Geneious platform offer tools for aligning next-generation sequence data chromatogram assembly, phylogenetics. Furthermore, it helps primer design, cloning and microsatellite analyses. Geneious also offers intuitive data management features, such as drag-and-drop file interface, local file directory organisation. It supports a wide range of files including FASTAQ format (**https://www.geneious.com**).

2.5.5. mtDNA-server

The mtDNA-server (Institute of Databases and Information Sytems (DBIS), University of Innsbruck) https://mtdna-server.uibk.ac.at/index.html, has a mtDNA database that contains mtDNA haplogroups that were used for classifying the samples into different haplogroups, and the results obtained were checked and confirmed using the phylogeny of global human mtDNA variations (which provides an online phylogenetic tree of global human mitochondrial DNA variation) on PhyloTree.org (Van Oven and Kayser, 2009). The complete workflow includes BAM format generation, heteroplasmy detection, contamination identification, interactive reports and the assignment of mtDNA haplogroups.

2.5.6. Haplogroup classification

Classification of mtDNA profiles into phylogenetic clusters (haplogroups) is mandatory in the area of phylogenetic and forensic genetics and is also applied in medical genetics. Availability of the NGS system has lead to an explosion of newly generated data. As a result, of the large increase of the published mtDNA sequences and the phylogenetic tree summarising them, the manual process of haplogroup classification would become time-consuming, and the need of an automatic way to address this issue is necessary. Several software and web services can offer this service, including HaploGrep, MitoTool and mtDNA server.

The MitoTool and HaploGrep web services have a mtDNA database that contains mtDNA haplogroups that were used in this study for classifying the various haplogroups to the samples and the results obtained were confirmed using the phylogeny of global human mtDNA variations (which provides an online phylogenetic tree of global human mitochondrial DNA variation) on PhyloTree.org 17 (Van Oven and Kayser, 2009). Following assignment of the haplogroups, data was compiled into an MSExcel spreadsheet, and the HVS-I of each group of samples that are belonging to the same haplogroup were used in FASTA format, which makes it easy to select samples for a particular network in .tor format.

2.5.7. HaploGrep software

HaploGrep is free software implemented as a web application, based on Phylotree. Using HaploGrep, it can automatically classify sequences into haplogroups. It supports a wide range of input format such as VCF and provides output formats like VCF, FASTA and multiple alignment formats. HaploGrep also offers useful tools for quality control (Figure 25). **(**https://haplogrep.uibk.ac.at) (Kloss- Brandstätter, 2011)

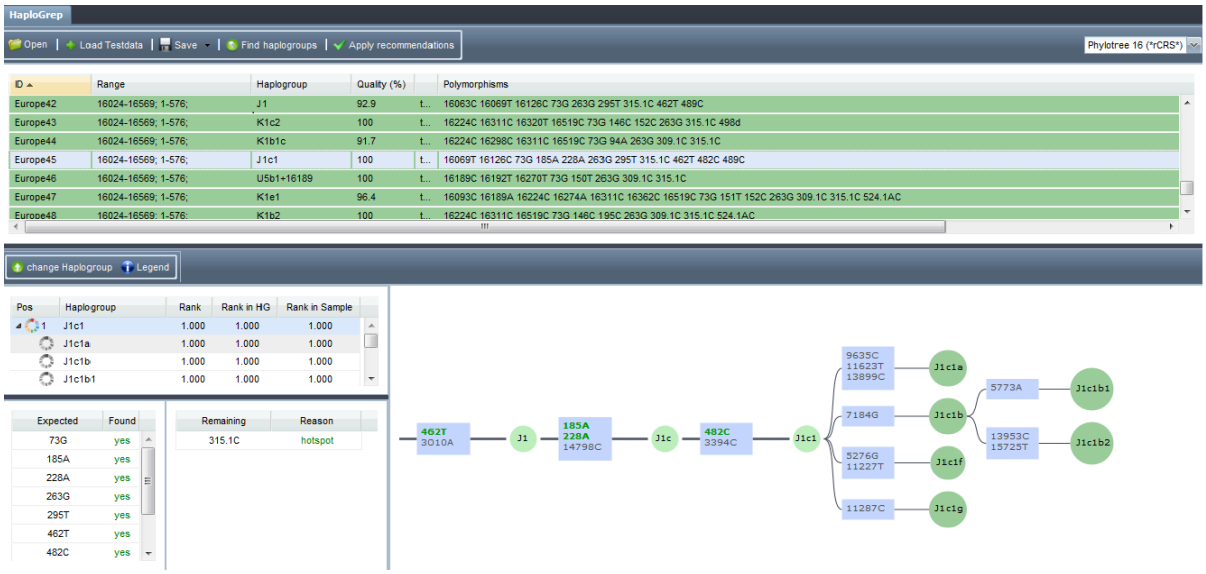

Figure 25. HaploGrep software interface

2.5.8. MitoTool

MitoTool is a web-based platform that, provides a convenient, interface for analysing human mtDNA sequence data. It automatically yields a list of the variants relative to the revised Cambridge Reference Sequence (rCRS) and determines the haplogroup status of that lineage based on Phylotree (www.phylotree.org). It also detects missing sequence variants in certain mtDNA sequences with claimed haplogroup status Figure 26 (**http://www.mitotool.org**).

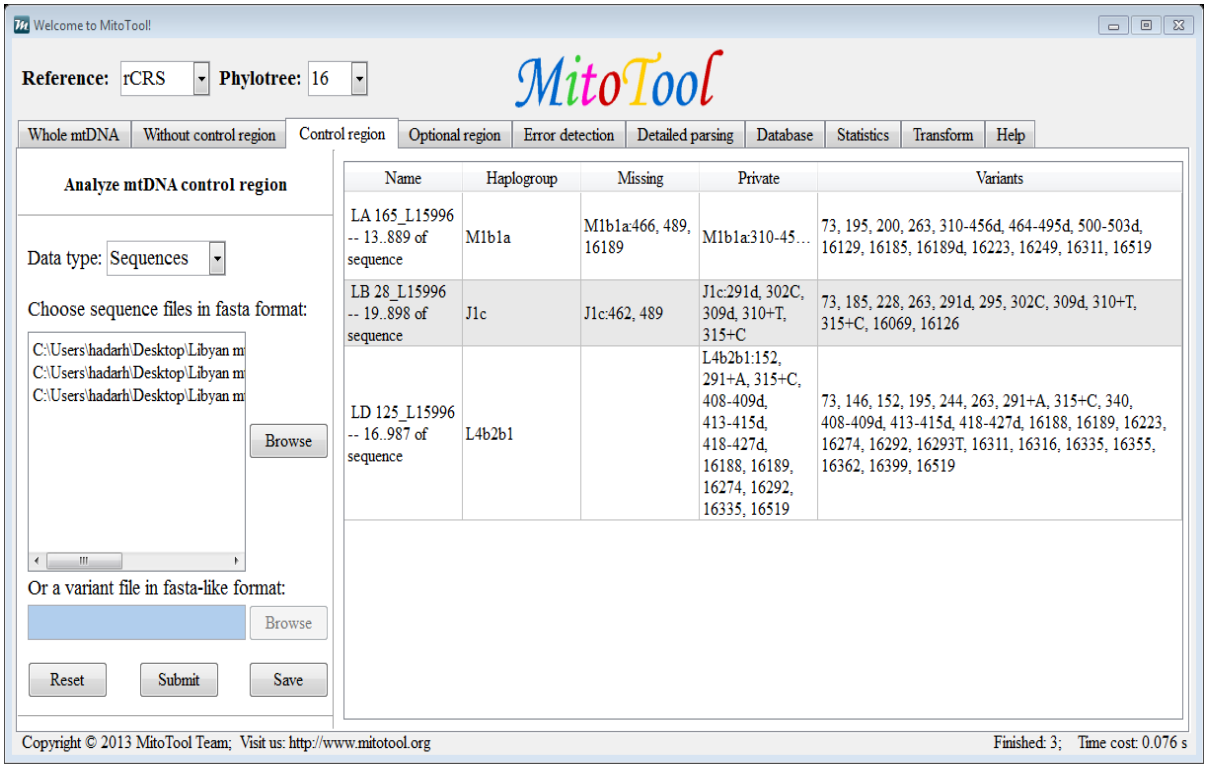

Figure 26. MitoTool interface

2.5.9. The 'fm2net_gui.'

This software introduced by Christopher Snell (Snell, 2008), is used to produce a.Tor file' that can be imported into FASTA format, which makes it easy to select samples for a specific network to build their phylogenetic tree using Network v5.0.0.0. software.

2.5.10. **Network Software**

The Network software v5.0.0.0 http://www.fluxus-engineering.com/nwpubterms.htm uses the maximum parsimony method to reconstruct the shortest least complex phylogenetic trees from the user data set. The network is built either used reduced median (RM) algorithm that needs binary data (example: at nucleotide position 16069 each taxon must have either C or T) or the median-joining (MJ) network algorithm if multi-state data (example: at nucleotide position 16092 there can be G, A, T, C, and ambiguities such as N) (Bandelt et al., 1995). This software uses HVS-I variants to link the haplotypes through nodes. This was calculated using the reduced Median and Medium joint algorithm. In the latter software, each haplotype was represented by binary data (0 or 1); 0 for absence, 1 for the presence of a variant and then saved as a ".rdf file". The rdf converter program Soares et al. (2009) then allows the nodes of the network to be automatically colour coded according to ethnic groups or geographic locations.

2.5.11. mtPhyl software

mtPhyl software reconstructs a maximum parsimony phylogenetic tree (Eltsov and Volodko, 2009). The software supports DNA sequence in a standard format like FASTA or ".txt" files containing partial or whole nucleotide sequence. The resulted tree can export into Microsoft PowerPoint or Excel formats. It can also estimate haplogroup divergence ρ (Morral et al., 1994; Saillard et al., 2000) using different mutation rates (Forster et al., 1996; Kivisild et al., 2006; Mishmar et al., 2003). mtPhyl allows users to specify mutations they want to use in phylogenetic tree reconstruction **(**https://sites.google.com/site/mtphyl/home**).** The molecular clock used in this study takes into account all substitutions and indels except the 16183C, 16182C, 16194C and 16519 (Soares et al., 2009).

2.5.12. Founder analysis

The purpose of conducting a founder analysis for mtDNA sequencing is to identify and date migrations. Founder analysis is software developed by Pedro Soares (Soares et al., 2009), using a

statistical calculation to assesses founder events and dates them using rho statistics that was first described by Morral et al. (1994) and then Forster et al. (1996). In this analysis, the lineages deriving from the potential founder and assumed source are dated, in the area under study. The time is estimated by using the diversity occurring and accumulated in the sink population, which is done using statistic ρ (Soares et al., 2009). The mutation rate used to perform. The founder analysis is one mutation every 2700 years (Soares et al., 2009). Thus the analysis provides a picture of the genetic contribution of immigration events to the current mtDNA pool throughout time. Furthermore, the method is formalised by applying a strict division between the potential source and sink populations. Also, the analysis takes in the account the homoplasy, back-migrations and recurrent mutation by using *f1* and *f2* for identifying founder sequences; so the founders must have at least one (*f1*) or two (*f2*) derived branches in the source population (Macaulay and Richards, 2008).

2.5.13. Bayesian migration partition (BMP).

This software is also a founder analysis, developed by Dr Soares group at the University of Minho Portugal, able to divide the founder lineages into migration periods (Richards et al. 2000). According to the (*f1-f2*) results, there are four migration events were defined in this study and used with the known historical events to help fractionate the founder lineages into migration events. The results of this analysis allow to probabilistically attributed to each specific defined historical or migration events using the BMP software. Four migration models were found connected to the major historical events were defined and used in this study. (20 kya, 11kya, 6 kya, 1.3 kya). This f analysis was done by Dr P. Soares at the University of Minho Portugal.

2.5.14. Bayesian evolutionary analysis by sampling trees

Bayesian evolutionary analysis by sampling trees (BEAST) v1.10.1 software (Suchard et al. 2018) is a program for Bayesian analysis of molecular sequences using Markov chain Monte Carlo (MCMC) approach. MCMC approach has been developed to be able to compute large hierarchical

models for thousands of integrated parameters. Bayesian evolutionary analysis by sampling trees (BEAST) is a program using the strict or relaxed clock to measure the change in population size over time starting from the roots. The BEAST program is using a Bayesian statistic to test the hypothesis of evolutionary models from a sample of gene sequences. The program can perform multiple estimations in a single analysis. It is able to estimate the substitution model parameters and calculate the evolutionary rate. Moreover, the program can infer the ancestral population dynamics and reconstruct demographic history under different expected scenarios (Drummond and Rambaut, 2007). The BEAST software package includes a BEAUTi software (Suchard et al., 2018) which uses Nexus sequence format (contains the sequence ID and haplogroup classification) that is created by Sequencher software. BEAUTi grouped the sequences monophyletically using sequence ID and haplogroup to finally create the input file XML to be run in BEAST to calculate a Bayesian skyline plot (BSP). The BEAST analysis was performed using a relaxed molecular clock to allow different molecular clock rates for different clades. Also, the HKY mutation model was used with gamma-distributed rates that allow different rates between transitions and transversions and variable base frequencies.

The length of the chain used in this study is 100,000000 iteration for each MCMC sample, screened every 1000 steps. BEAST software package also contains a Tracer v1.6 software (a graphical tool) that uses BEAST outputs files, a .log.txt and a .trees.txt to visualise Bayesian skyline plots (Rambaut et al., 2108).

2.6. Statistical Analysis

Population diversity parameters such as sequence diversity, number of unique sequences, nucleotide diversity or the mean pairwise differences of samples in the database were calculated using Arlequin 3.5 software (Excoffier et al., 2005).

Analyses of the molecular variance (AMOVAs) was also performed with the BioEdit Sequence Alignment Editor 7.2.5, Arlequin software, MEGA 5 and dnaSP v5 software were used to prepare sequences to be used in Arlequin 3.5 software. Neutrality tests Tajima's D and Fu's FS were used to testing the selection among Libyan groups were also performed using Arlequin 3.5 software

To define the genetic distance between the Libyan population and between Libyans and other population, multidimensional scale plot (MDS) of the mitochondrial and Y-chromosome lineages were performed. To analyse the relationships between different ethnic backgrounds in Libya, and between Libyans and other populations principal component analysis (PCA) was performed using MSExcel software implemented using absolute haplogroup frequencies using the XLSTAT software.

Haplogroup specific Median networks for haplogroups J, U, U6, K, L3b and L3f present in the dataset of the Libyan population were generated by the median-joining algorithm.

Time and age estimation was calculated using the rho described by Morral. et al (1994) and also developed by Forster et al., (1996), with one nucleotide substitution every 19,171 years for the HVSI sequences and 3624 for complete mtDNA sequences according to Soares et al., 2009. All the obtained ages were at least a 95% confidence interval.

Chapter 3

Mitochondrial DNA variations and maternal lineages landscape in Libya

3. Mitochondrial DNA variations and maternal lineages landscape in Libya

3.1. Introduction

As explained in the introduction chapter even though, the mtDNA database is growing rapidly; current mitochondrial DNA databases have limited representative data of the Libyan population. Only two studies have been done on mtDNA in the Libyan population; the first study is Ottoni's study (Ottoni et al., 2009) that analysed 129 Tuareg individuals from southern Libya. H1 comprised the main component in Libyan Tuareg at 61% followed by sub-Saharan lineages at 33%. The second study done by Fadhlaoui (Fadhlaoui- Zid et al., 2011b) characterised mtDNA HVSI in 269 Libyan individuals. Unfortunately, this study was done without discrimination of ethnicity or geographical origin with individuals just termed Libyan. The study found that West Eurasian lineages were the most predominant in Libyans found at 65%. sub-Saharan L lineages were found at the moderate percentage of 28%, and the indigenous lineages U6 and M1, combined were at 7%.

Therefore, this chapter aim to characterise the maternal genetic structure through analysis of the mitochondrial DNA HVI and HVII regions of 375 Libyan populations from different ethnic groups (175 Berber, 168 Arab, 32 African origin Libyan) and geographical locations and classify them into different haplogroups to identify the maternal ancestry of current Libyan population and infer their genetic position within wide world population and study their relationships to other North African, Arabian and sub-Saharan population populations.

Because not all haplogroups can be classified using mutations in the control region, or because of the presence of recurrent mutations, which can obscure the phylogenetic signature, the complete mtDNA genome was also amplified for the samples that could be not completely classified using control region data by Sanger sequencing the entire mtDNA in 18 overlapping PCR fragments. All the resulted samples were assigned to different haplogroups using HaploGrep and MitoTool software and confirmed using the phylogeny of global human mtDNA variations (which provides an online phylogenetic tree of global human mitochondrial DNA variation) on PhyloTree.org -

mtDNA tree Build 17 (18 Feb 2016), (Van Oven and Kayser, 2009). This was initially done by comparing to the rCRS motif (Anderson et al., 1989) using the criteria developed by Chen et al. (1995), Watson et al. (1997), Bandelt et al. (2001), Torroni et al. (2001) and Salas et al. (2002). Analyses of the molecular variance (AMOVAs), population diversity parameters sequence diversity, number of unique sequences, nucleotide diversity or the mean pairwise differences of samples in the database were calculated using Arlequin 3.5 software (Excoffier et al., 2005). Neutrality tests Tajima's D and Fu's FS were used to test the selectivity among Libyan groups. Finally, the genetic distance between the different Libyan population and between Libyans and other population were performed using multidimensional scale plot (MDS) and the relationships between different ethnic backgrounds in Libya, and between Libyans and other populations determined by principal component analysis (PCA) using absolute haplogroup frequencies.

3.2. Libyan maternal lineage landscape

The haplogroups distribution showed a similar picture to that has seen in North Africa (Fadhlaoui-Zid et al., 2004, Cherni et al., 2005, Fadhlaoui-Zid et al., 2011b, Aboukhalid et al., 2013, Badro et al., 2013, Bekada et al., 2013, Frigi et al., 2017a). The resulting sequence data for the four sampled ethnic groups are presented in Table 11 and Appendix Table 18. The Libyan mtDNA gene pool showed a dominance of Eurasian lineages (R0, HV, H, V, J, T. K, U. N1 and X) (63.8) Tables 11 and Appendix Table 18, Figures 27 and 28. West Eurasian haplogroups were present at 50.3% whilst other haplotypes mostly observed in Arabia (R0a, J1b, J1d and U7 12.7%. The second component is the Sub-Saharan lineages (West and East Africa) including L0, L1, L2, L3, and L4 at 30.8 %, reaching 75% in African Libyans, North African-specific sub-haplogroups U6 and M1 were found at 6.1% mainly in the Libyan Berber group (Figure 27).

Table 11. Haplogroup frequency among different Libyan groups

All Arabs include 168 Arabs in this study and 30 from Badro's study. All Libyans (this include 375 in this study, 129 Tuaregs (Ottoni et al., 2009) and 269 general Libyans (Fadhlaoui et al., 2011) and 30 Libyan Arabs (Badro et al., 2013).

Hg	Zuwara Berber (77)	Nafusa Berber (42)	Ghadames Berber (56)	All Berber (175)	Western Arab (125)	Eastern Arab (43)	All Arab (198)	African origin Libyan (32)	Libyan Tuareg (129)	This Study (375)	All Libyans (803)	Libyans without Tuareg (674)
L0	0	4.8	1.8	1.7	2.4	0	3	12.5	6.2	2.6	3.1	2.5
L1	1.3	0	1.8	1.1	5.6	7	5.1	12.5	3.9	4.3	3.7	3.7
L2	5.2	4.8	14.3	8	5.6	0	4.5	0	11.6	5.6	7.5	6.8
L3	2.6	14.3	19.6	10.9	18.4	21	20.2	46.9	11.6	17.6	15.4	16.2
L4	0	0	0	0	0.8	2.3	1	3.1	0	0.8	1.1	1.3
R0a	0	4.8	5.4	2.9	6.4	0	5.1	0	0	3.5	4	4.7
HV	6.5	9.5	1.8	5.7	11.2	0	7.1	0	0	6.4	5.9	7
V	0	0	0	0	1.6	0	1	0	3.9	0.5	0.9	0.3
H	16.9	26.2	8.9	16.6	24.8	25.6	25.3	12.5	61.2	20	26	19.1
J1b	0	0	5.4	1.7	1.6	4.7	2.5	0	0	1.9	1.6	1.9
J1c	32.4	0	0	14.3	0	0	0	0	0	6.7	3.4	4
J1d	0	0	10.7	3.4	0	9.3	2	0	0	2.7	1.2	1.5
J2	0	0	0	0	0	7	1.5	0	0	0.8	2.7	3.3
T1	3.9	0	0	1.7	0	0	0	0	0	0.8	0.9	1
T2	0	0	5.4	1.7	2.4	7	4.5	0	0	2.4	3	3.6
U	3.9	0	0	2.3	4	0	4	0	0	2.4	2.4	2.8
U5	2.6	7.1	3.6	4.6	0	0	0	0	0	1.9	2	2.4
U6	7.8	2.4	10.7	7.4	0.8	0	0.5	0	0	3.7	3.1	3.7
U7	0	0	0	0	3.2	0	2	0	0	1.1	0.5	0.6
K	11.7	21.4	5.4	12	4.8	9.3	5.1	0	0	8.3	5.6	6.7
M1	2.6	4.8	1.8	2.3	2.4	0	1.5	3.1	1.6	2.4	2.3	2.4
N	1.3	0	3.6	1.7	3.2	7	3.5	9.4	0	3.5	2.3	2.7
X	1.3	0	0	0.6	0.8	0	0.5	0	0	0.5	1	1.2

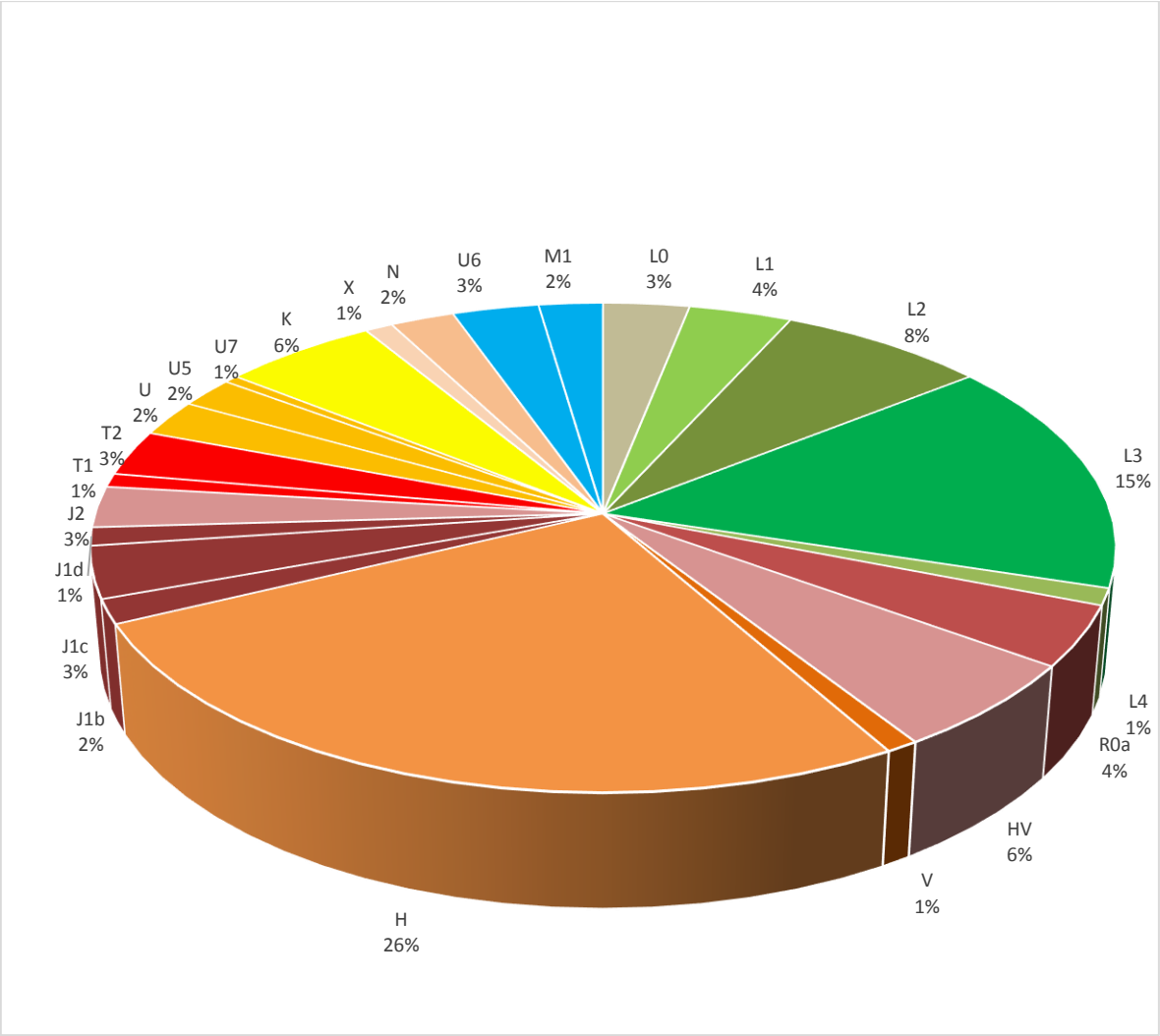

Figure 27. Relative frequency of mtDNA among the Libyan population

H and L3 haplogroups are the most frequent haplogroups among the Libyan population, at 26% and 15% respectively in Figure 28, 29). The haplogroup frequencies of the samples are presented in Table 11, and Appendix Table 18. The distribution of haplogroups varied among the different ethnic and geographic groups in Libya (Figure 29). Some of them were more frequent among Arabs and others more frequently in Berber or African Libyans. Arabian haplogroups J1b, J1d, J2 and U7, were only seen in Arabic groups and the Ghadames population (Figure 29). Furthermore, the highest frequency of this clade seen among the Eastern Arab population. In another hand, European haplogroups J1c, U5 and T1 were only found in Berber groups. Also, the vast majority of North African component (back Africa haplogroup U6 seen among Berber populations. A sub-Saharan

component haplogroups (L0, L1, L3 and L4) represents the vast majority of African Libyan Table 11 and Figure 29.

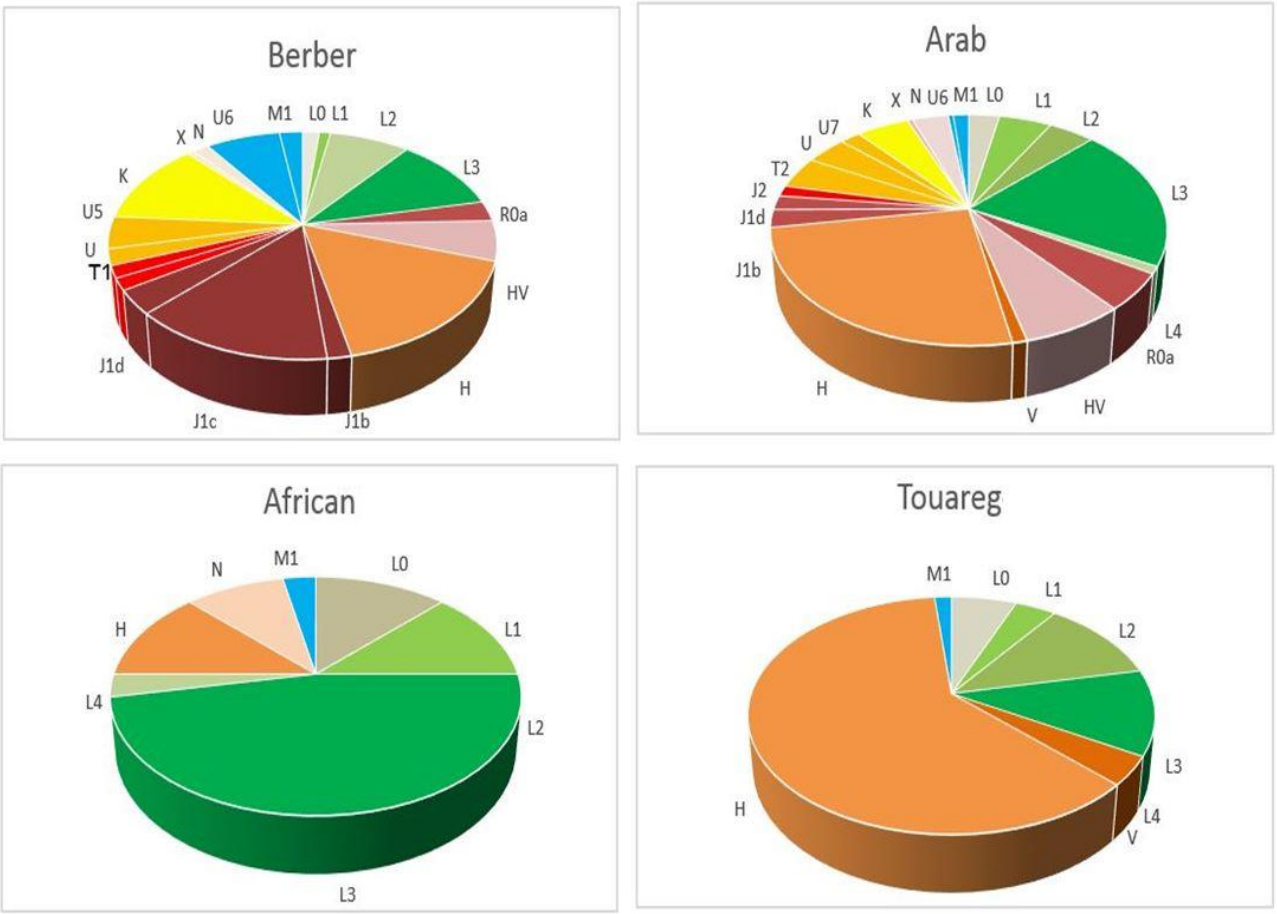

Figure 28. Relative frequency of mtDNA haplogroups among Libyan ethnic groups

Moreover, the result showed differences in haplogroup distribution among Berber populations. While Zuwara population characterized by high frequency of Eurasian clade J1c (32%), this clade not found among other Berber populations. Also Nafusa Berber characterized by high frequency of Eurasian haplogroups H (26%) and K (21%). On the other hand, the southern Berber Ghadames characterized by relatively high frequency of sub-Saharan lineages L2 (14%) and L3 (19.6) and the Arabian lineages J1b, J1d and R0a (5.4, 10.7 and 5.4) respectively (Figure 29).

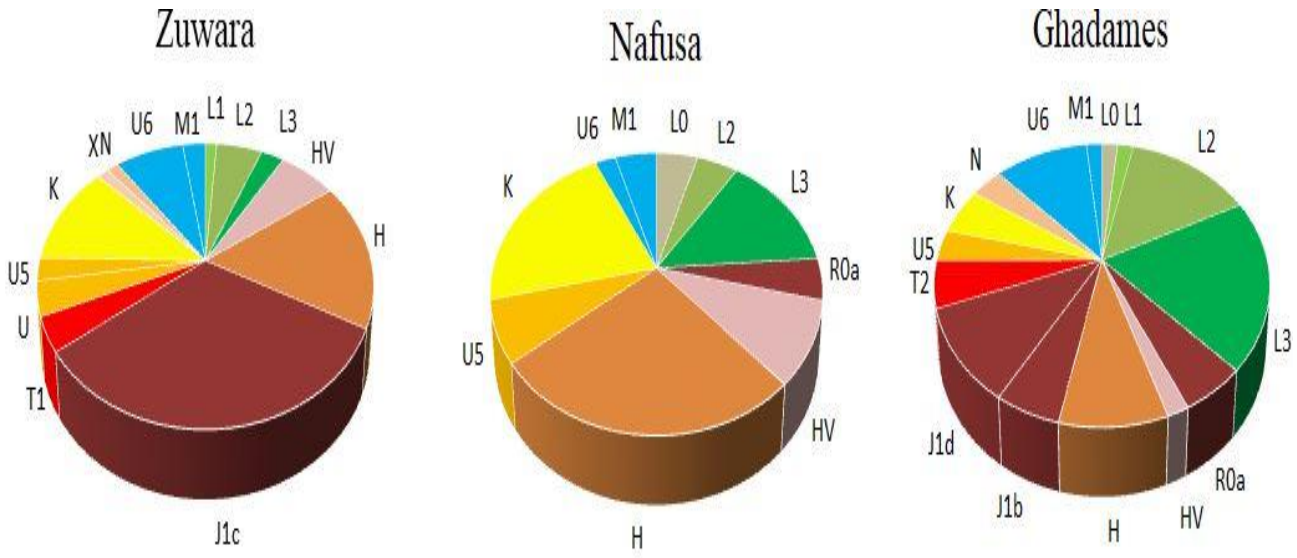

Figure 29. Relative frequency of mtDNA haplogroups among Berber speaking populations in Libya

3.2.1. Mitochondrial DNA gene diversity in the Libyan population

The sequence diversity analysis showed that the Libyan population as a whole present a higher diversity (0.998) in this study and (0.988) Fadhlaoui's study, than the average observed for other North African populations. (0.941) Table 12, (Coudray et al., 2009). However, the diversity among Libyan Berbers from Zuwara and Nafusa, and the Tuareg (Ottoni et al., 2009) are 0.804, and 0.823 0.677 respectively which is similar to other North African Berber. Interestingly when the 3 Berber populations considered as one group, the diversity index is dramatically increased by 0.987. Although African Libyan gene pool is constituted mainly by sub-Saharan lineages, the haplotype diversity is relatively high with a value of 0.984 Table 12.

Table 12. Summary of molecular diversity indices of Libyan populations compared with other relevant populations

Indices of molecular diversity in population from Africa, Europe and the Middle East, calculated from HVS-I haplotypes of the population collected from the published papers. Abbreviations are as follows: N, sample size; H, haplotype diversity; π, Nucleotide diversity; D, mean number of pairwise differences and SE for standard error.

Population	N	H	SE	Π	SE	D	SE	Reference
Libyan Tuareg	129	0.677	0.046	0.0115	0.0064	4.131	2.068	Ottoni et al., 2009
Takrouna	33	0.789	0.059	0.006	0.003	2.272	1.28	S. Frigi, et al. 2017
Zuwara Berber	77	0.8043	0.0554	0.0057	0.0035	2.4258	1.351	This study
Nafusa Berber	42	0.8238	0.06	0.0069	0.0042	2.9238	1.5963	This study
Jerbian Arab	29	0.904	0.022	0.011	0.0062	3.948	2.998	Cherni et al., 2005
Siwa Berber	78	0.914	0.014	0.0151	0.0081	5.436	2.644	Coudray et al., 2009
Kesra Berber	90	0.931	0.021	0.0174	0.0093	6.264	3.022	Cherni et al., 2005
Skhira Berber	20	0.937	0.043	0.0118	0.0068	4.237	2.185	Cherni et al., 2009
Figuig Berber	94	0.937	0.014	0.0171	0.0091	6.173	2.95	Coudray et al., 2009
Chnenni- Berber	53	0.939	0.017	0.0189	0.01	6.823	3.259	Fadahlaoui-Zid et al., 2004
Mozabites Berber	85	0.942	0.01	0.0134	0.0073	4.822	2.357	Macaulay et al., 1999
Kalaat El Andalous	29	0.946	0.024	0.012	0.007	4.493	2.278	Cherni et al., 2009
Matmata Berber	49	0.946	0.021	0.014	0.007	5.05	2.494	Fadahlaoui-Zid et al., 2004
Testour	50	0.958	0.021	0.016	0.009	5.783	2.814	Cherni et al., 2009
Kalat Al alia	48	0.96	0.016	0.015	0.008	5.507	2.695	Plaza et al., 2003
Souss Berber	50	0.961	0.018	0.0128	0.0071	4.604	2.295	Brakez et al., 2001
Asni Berber	53	0.963	0.016	0.0151	0.0082	5.424	2.65	Coudray et al., 2009
Bouhria Berber	70	0.964	0.011	0.0157	0.0084	5.661	2.744	Coudray et al., 2009
Portuguese	1591	0.965	0.003	0.01402	0.014	4.74	6.4	Barral-Arca et al. 2017
Algerians	47	0.965	0.012	0.0164	0.0088	5.894	2.861	Plaza et al., 2003
Moroccan Berber	64	0.968	0.032	0.0125	0.0069	4.52	5.48	Plaza et al., 2003
Slouguia	28	0.971	0.018	0.015	0.008	5.254	2.618	Cherni et al., 2009
Andalusia	1253	0.972	0.003	0.0128	0.0004	4.37	5.45	Barral-Arca al., 2016
Sened Berber	53	0.975	0.01	0.021	0.011	7.526	3.57	Fadahlaoui-Zid et al., 2004
Saharaui	56	0.976	0.012	0.0151	0.0082	5.448	2.659	Plaza et al., 2003
Jeriba	85	0.977	0.011	0.0153	0.0083	5.515	2.687	Cherni et al., 2009
Ghadames	56	0.978	0.0345	0.011	0.0065	4.6923	2.444	This study
Sejnane	47	0.978	0.011	0.017	0.009	6.14	2.973	Plaza et al., 2003
Mauritanians	64	0.979	0.008	0.0178	0.0095	6.407	3.071	Plaza et al., 2003
Western Arab	125	0.9795	0.0081	0.0212	0.011	9.1126	4.284	This study
Eastern Arab	43	0.9802	0.0176	0.0203	0.018	8.6443	4.143	This study
Jerbian Berber	30	0.983	0.016	0.013	0.007	5.018	2.508	Loueslati et al., 2006
Kurdistan	53	0.9833	0.0095	0.0192	0.0105	5.31	2.6046	McEvoy et al. 2004
African Libyan	32	0.9848	0.0403	0.0098	0.0059	4.1364	2.213	This study
Turkey	290	0.9851	0.0039	0.0174	0.0094	4.796	2.3499	McEvoy et al. 2004
Kuwait	94	0.9863	0.0051	0.0191	0.0103	5.273	2.5708	(Theyab et al., 2012)
Libyan	269	0.988	0.003	0.0189	0.0099	6.746	3.189	Fadahlaoui-Zid et al., 2011
Libyan	375	0.998	0.0001	0.0225	0.0131	8.456	4.356	This study
Syria	69	0.9881	0.007	0.0192	0.0104	5.307	2.594	McEvoy et al. 2004
Iran	92	0.9895	0.0049	0.0205	0.011	5.645	2.7325	McEvoy et al. 2004
Saudian	15	0.9905	0.0281	0.0222	0.0126	6.114	3.0821	(Abu-Amero et al., 2008)
Iraq	116	0.9918	0.0036	0.0199	0.0107	5.482	2.6568	McEvoy et al. 2004
Tunis Urban	98	0.992	0.004	0.0172	0.0094	6.433	3.3.070	Fadahlaoui-Zid et al., 2011
Egyptian	344	0.993	0.002	0.019	0.0099	6.832	3.224	Fadahlaoui-Zid et al., 2011
Moroccan Arab	50	0.993	0.007	0.0195	0.0103	7.04	2.96	Plaza et al., 2003
Nigeria	63	0.9949	0.0039	0.0239	0.0127	6.582	3.1513	Watson et al. 1996
Sudanese	76	0.995	0.003	0.0236	0.0122	8.482	3.963	Krings et al., 1999
Kenya	78	0.996	0.0033	0.0324	0.0167	8.903	4.1474	Watson et al. 1996

Compared with the rest Libyan groups and other North African Berber, the level of diversity observed in the Libyan Arabic groups is above the average level of mtDNA sequence diversity found in North Africa 0.979 and 0.980 Table 13. Sequence diversity was also quite high among the Ghadames population and African Libyan being 0.978 and 0.984 respectively Table 13.

The nucleotide diversity results showed the similarity between, Western and Eastern Arab as well as between Nafusa and Zuwara Berber, while the Ghadames population and African Libyan were in the middle position between other groups (Table 13). Furthermore, Nafusa Mountain Berber and coastal Zuwara Berber show similarity with other North African Berber Table 13.

Overall diversity amongst the Libyan population is high, where within 375 samples, there were 241 different haplotypes found. Within the same ethnic group, populations exhibit similar haplotype and nucleotide diversity values. The levels of genetic variation in Berber populations are comparable to the other North African Berber, and Libyan diversity in general comparable with other populations from North Africa and Arabia Table 13. Comparison of the genetic distance of the Libyan populations using FST values of pairs of the population is shown in Table 14. This demonstrates that the Libyan populations displayed significant differences in mtDNA variance Table 14.

Table 13. Pairwise FST values between Libyan populations

	Nafusa Berber	Zuwara Berber	African Libyan	Western Arab	Eastern Arab	Ghadames Berber
Nafusa Berber	0					
Zuwara Berber	0.01442	0				
African Libyan	0.04020	0.01005	0			
Western Arab	0.02729	0.02929	0.02624	0		
Eastern Arab	0.00400	0.01366	0.01705	0.01006	0	
Ghadames Berber	0.02370	0.00786	0.05217	0.05253	0.04559	0

Analysis of molecular variance (AMOVA) analysis among these Libyan populations and groups showed that profound genetic variations were accounted within each population (97.3%) than among populations (2.6%). While variance distribution was higher among groups (1.5%) than among populations within groups (1.15%) Table 14.

Table 14. AMOVA of mtDNA data in Libyan populations, grouped by ethnic groups using HVS and complete sequences

Source of Variation	Sum of squares	Variance components	Percentage Variation	F-Statistics
Among groups	1021.608	3.90292	1.50993	FCT: 0.01510
Among populations within groups	986.568	2.96305	1.14632	FSC: 0.01164
Within-population	41265.348	251.61798	97.34375	FST: 0.02656
Total	43273.524	258.48395		
Complete sequence				
Among groups	38450.547	148.282	3.17	FCT: 0.03168
Among populations within groups	27997.457	170.351	3.64	FSC: 0.03758
Within population	863719.193	4362.218	93.19	FST: 0.06807
Total	930167.196	4680.852		

3.2.2. Neutrality test

This test is done to evaluate if the current level of variation in the Libyan mtDNA gene pool is affected by selective forces, recent population expansion or shrink and genetic drift. Tajima's D and Fu's Fs tests are used to comparing population growths against constant population size. Tajima's D (Tajima, 1989) that is based on the infinite-site model is appropriate for use with non-recombination short DNA sequences to determine the neutral equilibrium model.

The result obtained for the neutrality test results were different from other North Africa populations, Tajima's D value was found to be positive in all Libyan populations Table 15. Fu's Fs value which is more sensitive to population changes was also found to be positive in all population when the populations were analysed individually except for Western Arab where the Fu's Fs value was found with a negative value. It also became negative when the Arabic and Berber populations were considered as groups and when all Libyan groups were mixed Table 15.

Positive values of these tests in the small population size indicate that populations underwent genetic bottlenecks. Also in the case of balancing selection, when the mutations are kept at intermediate frequencies, there will be more pairwise differences than segregating sites which produces a positive Tajima's D and Fu's FS values. On the contrary, negative values for this tests

indicate that the population expansion is under positive selection and doesn't have any demographic changes going such as migration. During population growth, this accumulates rare mutations, which also would lead to negative Tajima' D value and indicates recent population expansion (Ramírez-Soriano et al., 2008). The Libyan population harbour a negative Fu's FS value (-17. 087) and high mean genetic diversity (0.998) exhibiting strong population expansion mainly led by the Arabic group. Overall, although the neutrality test provided some evidence of recent population expansion in the Libyan population as a whole, the neutrality values obtained for groups, and the population within the groups are statistically not significant.

Table 15.Mitochondrial DNA neutrality measures in Libyan population groups and other relevant populations

Population	No	Tajima's D	P	Fus Fs	P	Sources
All Libyans	375	1.2374	0.098	-17.087	0.004	This study
Libyan Arab	168	9.229	0.76	-5.499	0.157	This study
Western Arab	125	8.719	0.065	-4.116	0.176	This study
All Libyan Berber	175	8.315	0.056	-1.2031	0.627	This study
Eastern Arab	43	6.787	0.095	0.2257	0.567	This study
Nafusa Berber	42	6.395	0.86	0.6657	0.687	This study
Ghadames Berber	56	5.188	0.0987	1.5699	0.829	This study
African Libyan	32	5.393	0.086	1.969	0.889	This study
Zuwara Berber	77	6.995	0.066	2.7607	0.957	This study
Takrouna Berber	33	−1.411	0.07	−1.475	0.233	Frigi, et al.2017
Slouguia	28	−1.472	0.055	−10.159	0	Cherni et al., 2009
Al Alalia	48	−1.385	0.06	−12.910	0	Plaza et al., 2003
Jerbian Arab	29	−1.587	0.042	−14.127	0	Cherni et al., 2005
Jeriba Berber	85	−1.470	0.04	−16.317	0	Cherni et al., 2009
Matmata Berber	49	−2.093	0.002	−17.203	0	Fadahlaoui-Zid et al., 2004
Jerbian Berber	30	−2.069	0.04	−22.554	0	Loueslati et al., 2006
Sened Berber	53	−1.635	0.024	−22.603	0	Fadahlaoui-Zid et al., 2004
Sejnane	47	−1.552	0.022	−23.959	0	S. Frigi, et al.2017
Kesra Berber	90	−1.536	0.029	−24.334	0	Cherni et al., 2005
Testour	50	−1.867	0.008	−25.269	0	Cherni et al., 2009
Chnenni- Douirt Berber	53	−0.882	0.181	−5.114	0.05	Fadahlaoui-Zid et al., 2004
Skhira Berber	20	−1.598	0.045	−6.234	0.003	Cherni et al., 2009
Kalaat El Andalous	29	−0.946	0.18	−6.559	0.003	Cherni et al., 2009

3.2.3. Mitochondrial DNA diversity in an ethnic and geographical context

In order to visualise the relationships between different Libyan populations in this study, multidimensional scaling (MDS) plots based on whole mtDNA genome data were performed from the pairwise Fst values between a pair of populations from Table 13. The MDS plot shows that

Eastern and Western Arab population are relatively close to each other on one side of the chart (Figure 30). Also, the Berbers from Nafusa and Ghadames were located in another half of the plot while the Zuwara Berber population were located at the same distance from the other Berber. The MDS plot also separates the African Libyan group from other Libyans revealing a clear differentiation between African Libyans and all other Libyan population. Berbers from Ghadames were also located on the edge of the plot showing differentiate away from other Libyan groups Figure 30. This distribution of different groups in Libya confirmed the diversity of the Libyan population.

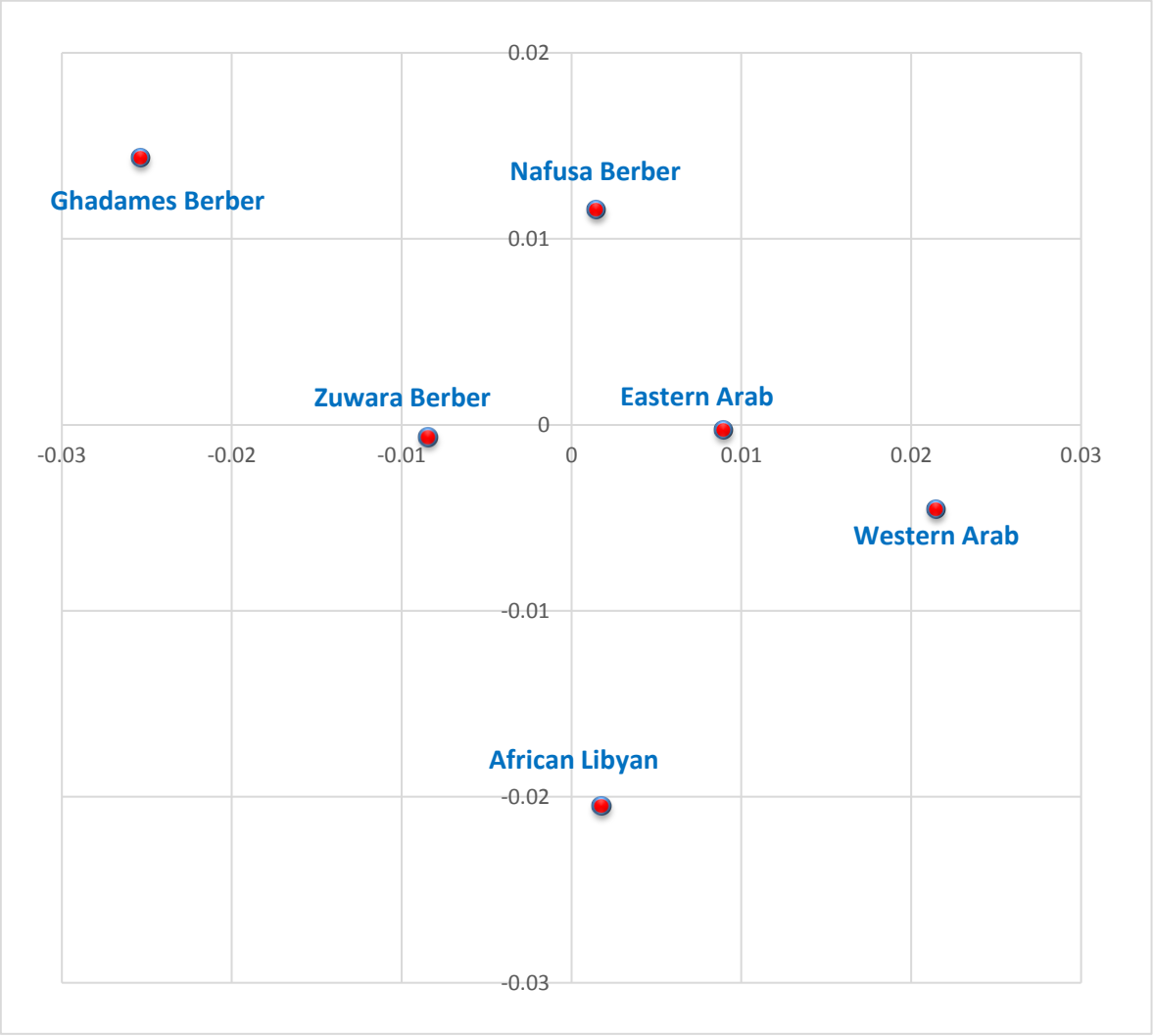

Figure 30. MDS plot based on pairwise Fst distance matrix among the different ethnic groups in Libya

To observe the maternal genetic relationship of the Libyan Berber populations with other North African Berbers, a principal component analysis (PCA) using the four Libyan populations who speak the Berber language (Zuwara, Nafusa and Ghadames in this study and Tuareg in Ottoni's study (Ottoni et al., 2009)) with 15 Berber populations from Tunisia, Algeria, Morocco and Egypt in addition to a Canary island population who speak Berber (Guanches).

PCA 1 and 2 (axes 1 and 2 in Figure 31) account for 71 68% of the total variation (60.98% and 10.70%, respectively). The two-dimensional pattern does not display any clear, distinct group clusters according to the geographical location of the population except for Siwa, an Egyptian Berber who show a clear genetic differentiation from other Berber population. However, the Ghadames population that is located on the western edge of the Libyan Sahara show proximity with Figuge Moroccan Berber and the Siwan population. All of these three Berber groups are located on the edge of Sahara. PCA analysis revealed that Zuwara coastal Libyan Berbers are located in the plot near the Chenini-Douiret Tunisian Berber, and Jerbian Berber who are described as the closest Berber populations to the Europeans (Frigi et al., 2017a). Nafusa mountain Berbers were located in the middle of all Berber population between Mozabite Algerian Berber and Souss Moroccan Berber and Sejnane and Takrouna Tunisian Berber. The last Libyan group the Tuareg were located at the edge of the plot near Asni and Bouhria Moroccan Berber and Guanches from the Canary Island characterised by a high percentage of haplogroup H.

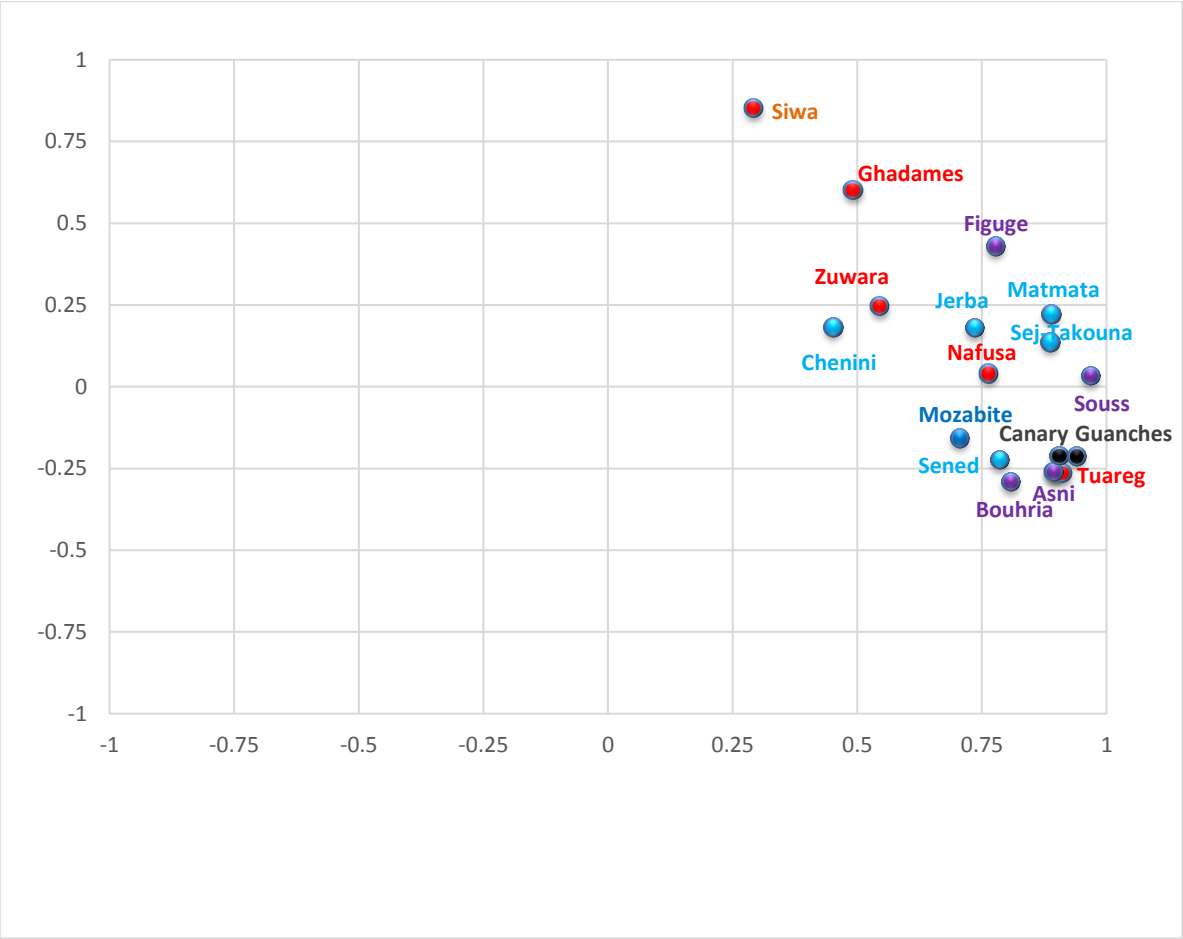

Figure 31. Results of the PCA of Libyan Berber speaking populations within a North African Berber landscape

The analysis was performed with the four Libyans populations speaking Berber in red and 13 additional Berber population; Tunisia in sky blue, Moroccan in purple, Egyptian in orange, Algerian in blue and Canarias in black.

To have a general view of the signal of gene flow in Libyan population, PC analysis was performed based on haplogroup frequency of 70 worldwide populations from Africa, Europe, Arabian, Iberian and Near East populations. PCA 1 and 2 (axes 1 and 2 in Figure 32) account for 69.14% of the total variation (55.72% and 13.42%, respectively). The populations clustered in the chart according to their geographical location. The first dimension separated the sub-Saharan population from the other populations which are characterised by having a higher proportion of haplogroup L. The second dimension separated Near Eastern population from the majority of the North African population.

European populations located on the edge of the chart is characterised by higher frequencies of H, and U haplogroups Figure 32. The Arabian Peninsula populations were located in the middle of the chart separated by the Near East and sub-Saharan populations. While Libyan Arab, Nafusa Berber and Libyan Tuareg are located within the North African population. The Ghadames population is located near the Siwan Egyptian Saharan Berber and Figuge Moroccan Saharan Berber.

The Zuwaran Berber population was located isolated from North Africa in an unexpected position with Chenini-Douiret Tunisian Berber between Levante and Arabian populations.

In general, the PCA plot separated the populations according to the geographical location of the population with some exceptions; the Libyans of African origin were located closed to Western African populations, while the Ghadames population showed more proximity to Siwan Egyptian Berber rather than other North African Berbers. Also, Zuwarans were located more close to Levant populations (Jordan and Syria). The results of the PCA for the Libyan Berbers therefore clearly demonstrated the close relation with other North African Berber population. In addition, the PCA plot clearly confirmed the Libyan affiliation to the other North African populations in Morocco, Algeria and Tunisia.

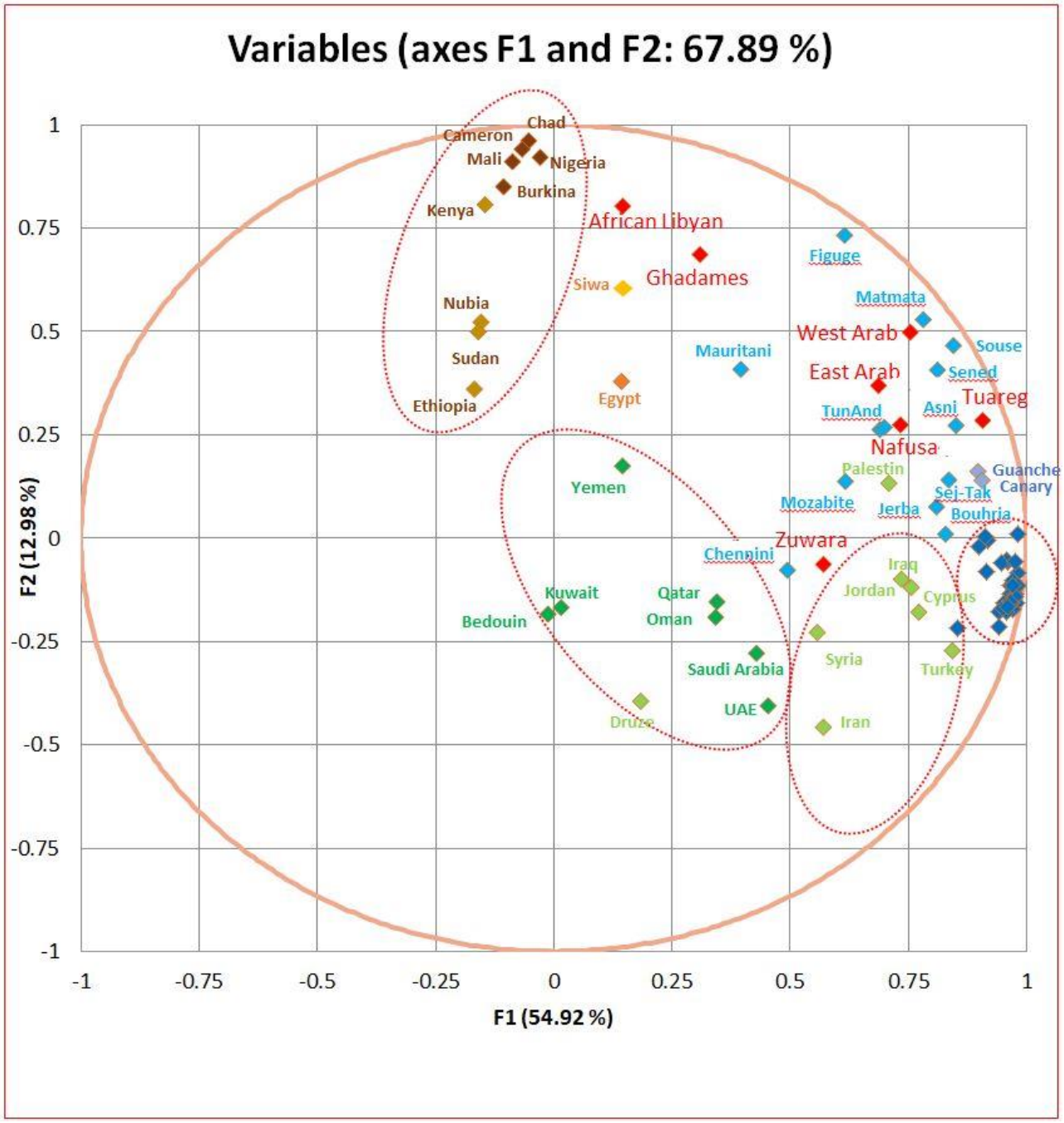

Figure 32. Results of the PCA performed with the Libyan group's data and worldwide based on haplogroup frequencies

PCA performed with Libyan groups in red and 70 worldwide population; North Africa in sky blue, West and East Africa in Brown, Europe in blue, Arabian in Green, Near East in light green, Egyptian in orange and Iberian in grey.
Abbreviations: Sej, Sejnan; Tak, Takrouna; UAE, Emirates; TunAnd, Tunisian Andalusia

In order to visualize the position of the Libyan mtDNA profile and to have a general view of the maternal genetic landscape, a PCA plot was constructed with the Libyan dataset and 63 worldwide population including Africa, Arabia, Near East and Europe. The two-dimensional pattern displays clear distinct clusters that show that worldwide populations were relatively grouped according to

geographical distribution. PCA 1 and 2 (axes 1 and 2 in Figure 33) account for 69.39 of the total variation (56.23% and 13.16%, respectively). The first dimension separates sub-Saharan population (West Africa; Cameron, Nigeria, Mali, Chad, and Burkina Faso and East Africa; Sudan, Ethiopia and Kenya) from the other populations characterised by haplogroup L, Figure 33. African populations were also clearly separated into West and East African populations. European populations are clustered on the opposite side to sub-Saharan African to edge of the chart characterised by H haplogroup. Near Eastern populations were located in the middle of the plot and were separated from the European populations and the Arabian peninsula populations. The second dimension separated European populations from North African populations. Yemen and Egypt showed genetic proximity and were located in the middle of North Africa, Arabia and East Africa population. The Libyan population was located in the Middle of the North African populations in the plot near the Moroccan population Figure 33.

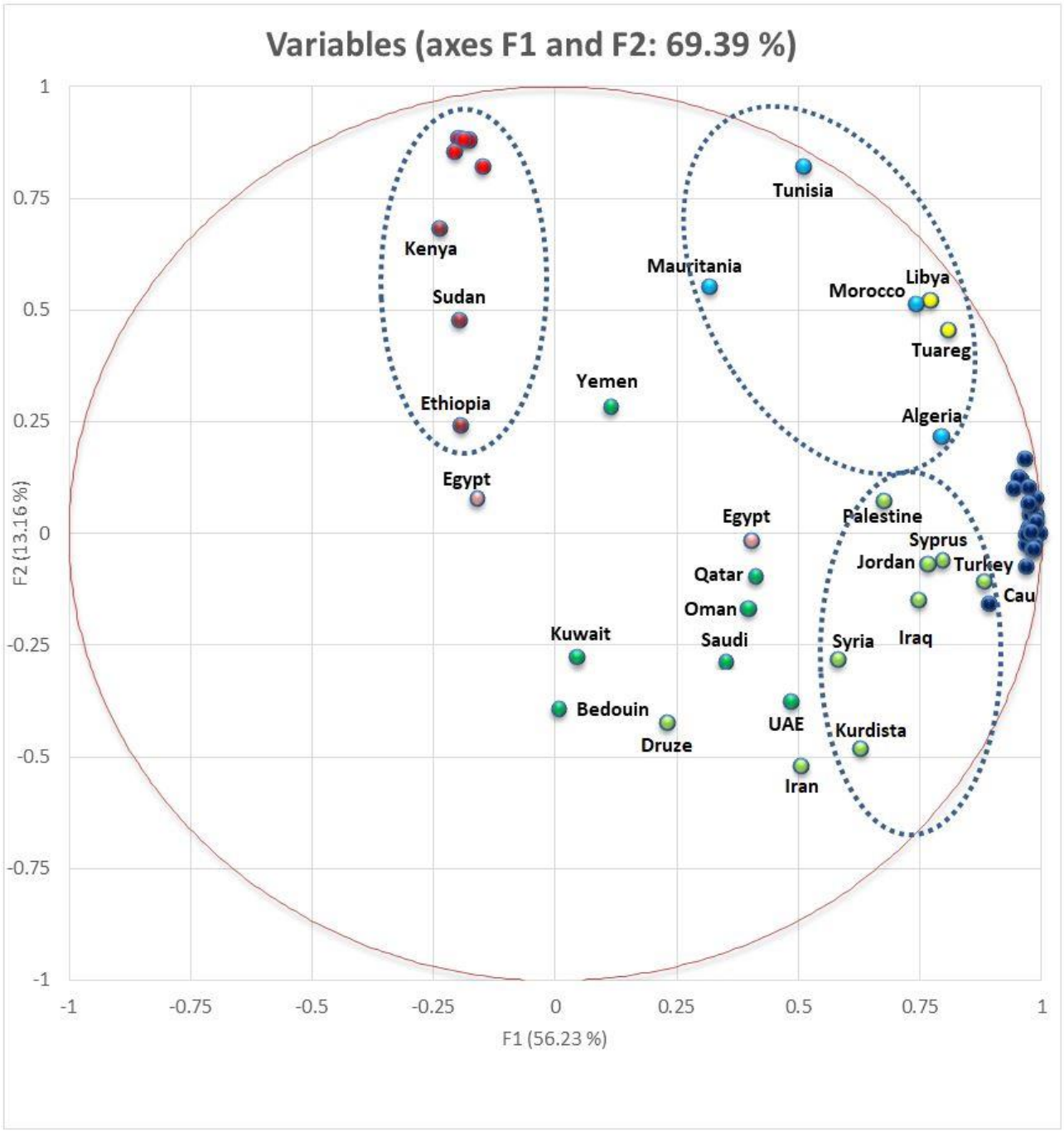

Figure 33. Results of the PCA performed with the Libyan population data and worldwide based on Haplogroup frequencies

PCA performed with Libyan population in yellow and 63 worldwide populations; North Africa in sky blue, West Africa in red, East Africa in brown, Europe in blue, Arabian in Green, Near East in light green and Egyptian in pink. Abbreviations: UAE, United Arab of Emirates: Cau, Caucuses.

3.2. **Discussion**

3.2.1. **Genetic diversity among the Libyan population**

The results of this study revealed the heterogeneity the Libyan group's mtDNA sequences and the different haplogroup distribution between the different Libyan populations. While African Libyan as expected were characterised by a high percentage (75%) of sub-Saharan lineage (L0 L1, L3, L4) pointing to the African origin of this population, West Eurasian haplogroups (N1, X, J, T, HV, H, U and K) accounted for 80% of those found among coastal Libyan Berbers from Zuwara. Nafusa Berbers were characterised by the high frequency of European haplogroups (H at 26.6% and K at 21.45%). Ghadames Saharan Berber and Western Arab groups were characterised by a variety of haplogroups. Ghadames population characterised by a high percentage of haplogroup L2 (14.3%). Among sub-Saharan lineages, L3 is the most abundant haplogroup being 17.6% and was 47% among African Libyan. The J1c clade is the most frequent sequence found in the Zuwaran Berber population reaching 32%. The finding of this study is compatible with the results obtained by Fadhlaoui and her colleagues (Fadhlaoui-Zid et al., 2011b) where they found that west Eurasian components are predominant in Libyans (65%), sub-Saharan L components found at moderate frequency (28%), and North African lineages U6 and M1, which have an overall frequency of 7%.

Furthermore, the results demonstrated a difference in the distribution of haplogroups and subclades among different ethnic and geographical groups in Libya. Typical North African group U6 and M1, and European haplogroups J1c, T1 and U5 were mainly restricted to Berber population confirming the relation of Libyan Berber to other North African Berber. In contrast, Arabian haplogroups J1b, J1d, J2 and U7 were mainly seen in Arabic groups and the Ghadames population and the highest frequency of this clade was seen among the Arabs who live in Eastern Part of Libya suggesting that the Eastern part of Libya received more gene flow from Levant and Arabian peninsula. Moreover, the study revealed differences among Berber population where the Ghadames gene pool showed

more proximity to other Saharan Berbers such as the Siwa in Egypt and Figuge in Morocco than the other Libyan and North African Berbers (Chaabani and Cox, 1988, Coudray et al., 2009, Fadhlaoui-Zid et al., 2004). There was also a degree of heterogeneity between coastal Berbers (Zuwara) and mountain Berbers (Nafusa) that reflects the heterogeneity among Libyan Berber. In the African group, sub-Saharan lineage L was the most abundant haplogroup. The presence of the West African lineages is probably a result of the major migration of the sub-Saharan populations, where the slave caravans came to the Southern region, Fezzan, in 1929, and then migrated to the coastal cities during the Second World War such as Zliten and Tuareg (Najem, 2004). Remarkably in the context of Libya, the haplogroup data present a picture that is quite complex for Tuareg population, where their mitochondrial gene pool is characterised by high percentage of European haplogroup H1 (61.2%) which explains the low diversity indices among Tuareg.

The overall gene pool picture of Libyan population confirmed the position of Libyan population among the other North African populations in an intermediate position between sub-Saharans, European and Arabia with a predominance of Eurasian haplogroups. The high diversity of Libyan population may refer to haplogroup assignment for the Libyan where the Libyan gene pool contains a wide range of major haplogroups including Eurasian, sub-Saharan, North African and Arabia as well as an increased frequency of East African and Arabian haplogroups comparing with other North African populations, reflecting the greater effect of both Near Eastern population expansions at the end of the Pleistocene and in the Holocene and by sub-Saharan populations that migrated in recent times. Among the Libyan groups in this study, the African origin Libyan seems to be the most genetically isolated from other Libyan groups with a high West sub-Saharan component. Zuwaran Berber were characterised by low diversity and a high West Eurasian component in their mtDNA gene pool, especially J1c clade.

Genetic diversity values pointed to the low gene diversity in Zuwara and Nafusa Berbers compared with the rest of the Libyan groups. The diversity indices obtained for Zuwara and Nafusa Mountain were 0.804 and 0.823 respectively while in other Libyan groups the diversity indices were 0.979

for Western Libyan Arab, 0.980 for Eastern Libyan Arab, 0.978 for Ghadames population and 0.984 for African Libyan, 0.985 for Libyan Tuareg (Ottoni et al., 2009).

The observed low diversity in the mtDNA gene pool of the Berbers from Zuwara and Nafusa population could be attributable to consanguinity and drift, as these particular populations live isolated from other Libyan populations. mtDNA markers are more sensitive to genetic drift, bottlenecks and founder events due to its effective size being 1/4 comparing to autosomal. These effects can be significant when samplings are carried out in isolated small towns like Zuwara (40000) where inbreeding is very strong. Comparison of the genetic distance of the Libyan population using FST values of pairs of population demonstrates that the Libyan population displayed differences in mtDNA variance. This finding indicates that even the population from the same ethnic background (Zuwara, Nafousa and Ghadames) had significant genetic differentiation that was reflected in the sample distribution in a multidimensional plot (MDS) Figure 34.

Taking into account the genetic diversity of different ethnic groups in Libya, namely Arab, Berber, Tuareg and African, this study found small variations between the populations within groups but relatively higher variations among groups. AMOVA was performed using both complete mitochondrial genome and control region data. The analysis showed that profound genetic variations were accounted within each population than among populations. While variance distribution was higher among groups (1.5%) than among population within groups (1.15%). This pattern of variation indicates that the genetic variation of some individuals is closer to individuals from another population or groups than individuals from the same population. The majority of Libyans who considering them self Arab are in fact a mixture of Berber indigenous and Arabs who migrated from the Arabian Peninsula. This was also confirmed by low frequencies of Arabian haplogroups such as U1, U3, U7 and N that are much more predominant in Arabia than elsewhere. Furthermore, although Ghadames is a small town, contain a small size of the population (18000) speaking Berber language, it can be inferred from the haplogroup distribution data that the

Ghadames population are a mixture of Berber, Arab and African origin populations that arrived in that town in different periods. Moreover, the variation appeared between groups as mentioned above seemed to be characteristic of haplogroup distributed among the Libyan groups, such as haplogroup L in African, H in Tuareg and U6, U5, T1, J1c among Berber group.

Fu's FS values were found to have a significant negative value (-17) among Libyan as a whole and less significant when populations were analysed as groups or as indiviual population. This result indicates the presence of population expansion under a bottleneck or a purifying selection. This also leads to an excess of rare polymorphisms. However, the Tajima's D values were positive which may reflect the different mutation patterns among the Libyan population, which finally increase the variation among the population. The positive the Tajima's D values are also indicated by homogenous variations among population within the groups with more differentiation between groups. Negative Fu's FS values accompanied by positive Tajima's D are explained by the presence of a recent population expansion led by the African and Arab groups (Ramírez-Soriano et al., 2008). However, the neutrality value for populations individually are statistically not significant and make these conclusions less strong.

The low level of diversity seen among Zuwara and Nafusa Berbers might signify it is because of low levels of both rare and common mutations which are a result of bottleneck or genetic drift (Ramírez-Soriano et al., 2008). Furthermore, mtDNA markers are more sensitive to genetic drift, bottlenecks and founder events. This explains a positive Tajima's D and Fu's FS values seen among Zuwara, Nafusa and Tuareg Berbers.

In conclusion, these data provide good information on the dispersal between the majority of Libyan ethnic groups and gives a good overall view of diversity, which is also valuable for forensic purposes.

3.2.2. **Libyan maternal lineage landscape among the neighbouring population**

When comparing the haplogroup distribution in Libya with another neighbouring populations, the results confirmed the Libyans' affiliation to North African populations and demonstrated the genetic differentiation with Eastern and sub-Saharan populations suggesting the presence of a barrier between Libya and Egypt as well as to Southern African populations, in addition to the Mediterranean sea that separates Libya from Southern Europe. Egyptian population studies revealed a different distribution of Eurasian lineage, where J haplogroup is predominant (76%), followed by T (9.4%) U (9%) and K (4.7%) (Saunier et al., 2009); moreover, the African lineage represents including 20.6% and Asia lineage including M and N is 11.9% (Saunier et al., 2009). This differentiation between Egypt and the North African populations has previously been mentioned by the Fadhaoui et al., 2011b. Fadhloaoui et al suggested the presence of more gene flow in the Nile River Valley coming from East Africa into Egypt that leads to different haplogroup frequency distributions of L, U6, M, I, W and H lineages. Sub-Saharan population are characterised by high percentage of L haplogroup 90% for West Africa and L and M haplogroup for East African population, (Cerezo et al., 2011, Pereira et al., 2010a, Afonso et al., 2008, Černý et al., 2007, González et al., 2006) suggesting that the Saharan barrier may lead to this differentiation between North Africa and sub-Saharan population. Although the gene pool of Libya and Northern Africa are characterised by a considerable percentage of haplogroup H (Ennafaa et al., 2009), (19% among Libyans) The South and South western part of Europe is constituted by more than 40% of H lineages. The differentiation in haplogroup distribution between Europe and North Africa, especially sub-Saharan L and back African M1 and U6 are referred to as the Mediterranean barrier (Ennafaa et al., 2009). Finally, even the majority of North Africa speak Arabic language which was brought by the Arab Muslims who migrated to the region to spread the Islam, Libyan haplogroups distribution are different from Arabia and Levant haplogroups seen in studies (Abu-Amero et al., 2007a, Abu-Amero et al., 2007b, Abu-Amero et al., 2008, Alshamali et al., 2008, Al-Zahery et al., 2013, González et al., 2008, Kivisild et al., 2004, Scheible et al., 2011, Vyas et al., 2016). This

suggests that the maternal gene pool in Libya was not widely affected by Arab expansion to the area and their role as the other North African populations were restricted to a cultural role (Fadhlaoui-Zid et al., 2011b, Ennafaa et al., 2009).

3.3.**Conclusion**

The conclusion from the results of this study revealed that the current Libyan gene pool is similar to other North African population constituted by various migrations and different genetic lineages from different geographical locations such as sub-Saharan Africa, Arabia, Near East and Southern Europe. This conclusion is also supported by the PCA carried out in this study revealed that Libya populations composition as a whole are similar to the components found in other North Africans populations than any other populations as previously reported by Fadhlaoui and her colleagues (Fadhlaoui-Zid et al., 2011b). Also, the PCA plot based on mitochondrial haplogroup frequencies reflects a clear differentiation between Libyan Berbers, where the plot put Ghadames in the positive portion of the first axis between Moroccan Berber Figuge and Siwa Berber. Even though there is the long distance between the three Berber-speaking pre-Saharan oases, Ghadames in Libya, Siwa in Egypt and Figuge in Morocco, the chart put them in the same position quite away from other North Africa Berbers, pointing a genetic similarity between them that is led by a relatively high percentage of L haplogroup. This may be due to Ghadames being a trade centre connecting Eastern and Western North Africa; it also connects North Africa and sub-Saharan Africa that is reflected by the high range of haplogroup diversity among the population.

Zuwara Berber were located in the same position in the PCA with Chenini and Douiret Tunisian Berber with some proximity with Jerbian Berber, isolated from other North African populations and were characterised by a high percentage of Eurasian components such as J1c, H and K (Ben Halim et al., 2018). Nafusa mountain Berber population showed typical North African Berber located in middle chart. As expected African origin Libyans showed more proximity to West African population characterised by high percentage of sub-Saharan components confirming the gene flow

of sub-Saharan lineage into Libya. Another component of the Arabized Blacks is the Tebu, which live on the Libyan border with Chad, Niger and Sudan (Winter and Koch, 1999, Van Waas, 2013b).

In conclusion, the current Libyan gene pool is constituted by mosaic lineages dominated by Eurasians haplogroups with a moderate percentage of sub-Saharan component followed by Arabian and back African haplogroups.

Neutrality tests Fu's FS and Tajima's D revealed the presence of recent population expansion led by Arabic group. It can also be inferred from FST pairwise difference between population the presence of significant genetic variations between populations. The significant difference between ethnic groups was revealed by AMOVA indicating the presence of multiple ancestors for Libyan population coexists with a weak genetic flow between different the four ethnic groups regardless of geographical location.

Overall, as the Libyan data in this study include the main ethnic groups and the most geographical locations in Libya that may serve as a base for comparison of Libyan populations with other populations and pave the way for the establishment of Libyan databases that can be used for the forensic casework.

Chapter 4

Results and discussions: Phylogeography of Libyan variations

4. Results and Discussion: Phylogeography of Libyan mtDNA variations

4.1. Introduction

The database of complete mtDNA is growing rapidly, however; the high-quality mtDNA database mainly contains West Eurasia and the Asian population's data. North African populations are still underrepresented (Turchi et al. 2009). The increasing the number of complete mtDNA sequences in the database has greatly improved the human mtDNA phylogenetic tree and offered an advanced understanding of the phylogeography of particular haplogroups (Torroni et al. 2006; Abu-Amero et al. 2007; Derenko et al. 2007; Soares et al. 2008). These studies aim to gain information about population history by using the age of haplogroups that estimated from the mutations that have accumulated along maternal line and their geographic distribution.

The aim of this part of the thesis was to analyse and describe complete mitochondrial DNA genome variations in the Libyan population. This chapter also aims to infer the possible source and estimate the time of arrival of different ethnic and geographical groups in Libya. Firstly the samples were screened by hypervariable sequencing segments of the mtDNA control region to classify haplogroups, and then representatives from each haplogroup (199 samples) were sequenced for complete mtDNA genome sequencing using NGS technique. 91 of these samples were from Berber individuals that included 16 samples from Nafusa Mountain Berber, 38 coastal Berbers from Zuwara and 37 Saharan Berbers from Ghadames. 90 samples were from Arabs, including 68 Western Libyan Arab, 22 Eastern Libyan Arab. In addition, 18 African origin Libyans were analysed.

Phylogenetic trees were constructed using mtPhyl software using maximum parsimony phylogenetic tree (https://sites.google.com/site/mtphyl/home). Time and age estimation was calculated using the rho described by Morral. et al (1994) and also developed by Forster et al., (1996). Founder analysis application for mtDNA sequencing (developed by Pedro Soares) was also performed to identify and date of migrations. Finally Bayesian skyline plots (BSP) of population

size change through time constructed for samples of mtDNA genome sequences (Rambaut and Drummond, 2007).

4.2. mtDNA Lineages in the Libyan population

The Libyan mtDNA gene pool (Figure 34) is constituted mainly by the Eurasian component (63%). The West Eurasian including N1, R, R0, U and X represented at 50.3% and other haplotypes observed were mostly Arabian R0a, J1b, J1d and U7 represented at 12.7% (Figure 35). The second component is the sub-Saharan lineages at a modest frequency (30.8 %). West and East Africa including L0, L1, L2, L3. North African-specific sub-haplogroups (back to Africa) U6 and M1 are found at (6.1%).

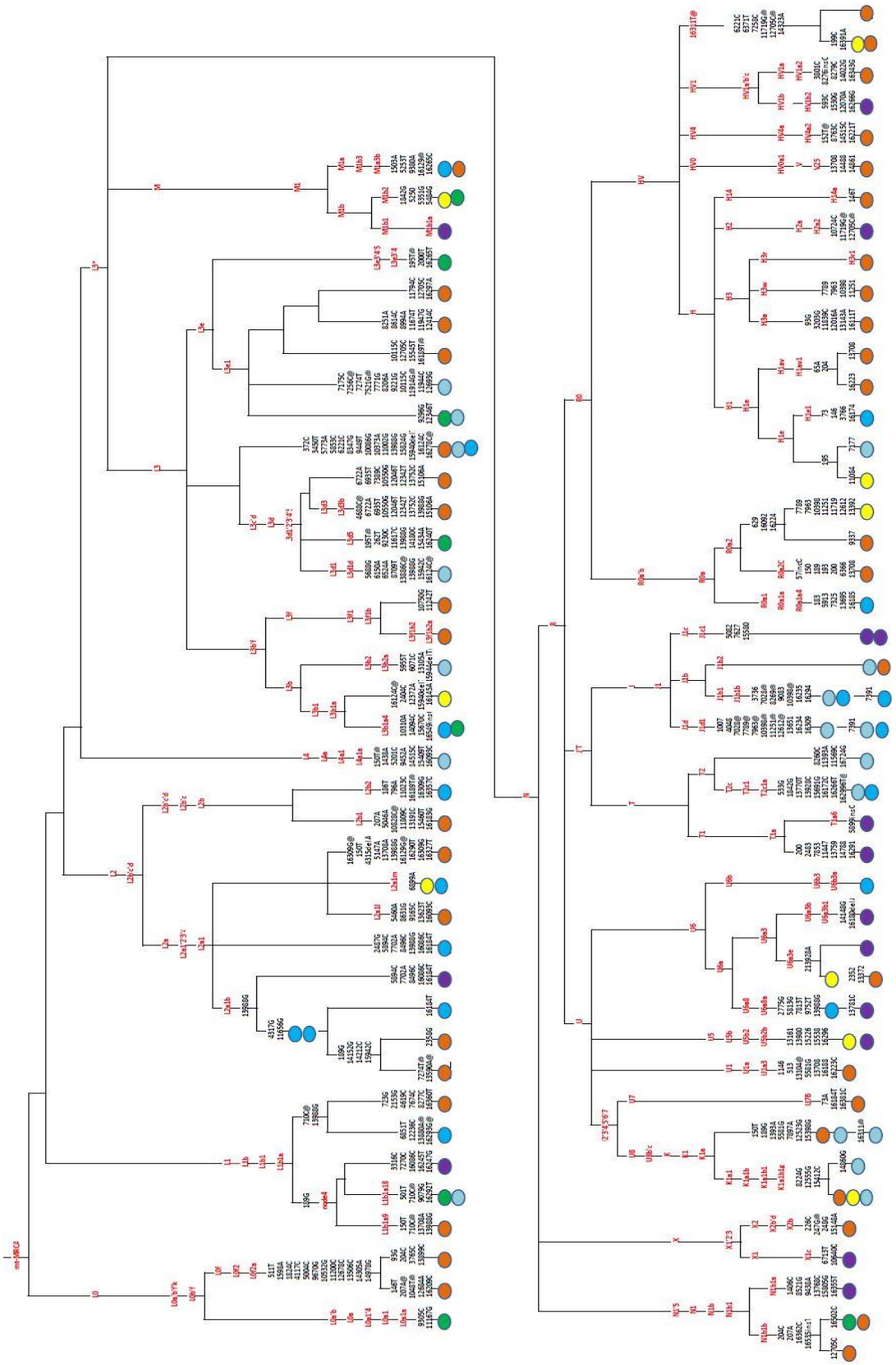

Figure 34. The tree of complete mtDNA lineages in Libyan population

Western Libyan Arab samples in orange, Eastern Libyan Arab samples in light blue, Zouara Berber in purple, Nafusa Berber in yellow, Ghadames population in blue and African Libyan in green.

4.3. Lineages of Eurasian origin in the Libyan population

The haplogroup N lineages in Libyan population are stratified into nine general haplogroups: N1, R0, HV, V, H, J, T, U, K, and X designated as the Eurasian haplogroups.

4.3.1. Haplogroup N1b

Eurasians haplogroup N originated at a geographical location between Southwest Asia and Australasia at ~50–65 kya, very close to the age of L3 clade in Africa (Fernandes et al., 2012). N1b branch of N has three basal branches: N1b1, N1b2, and N1b3 (Fernandes et al., 2012). N1b1 is the most abundant of the clades, that contain several branches that originated in the Near East, central and eastern Mediterranean Europe, Arabia, and Northern Africa that were dated to ~14 kya (Fernandes et al., 2012). These lineages were represented in Libya by N1b1b clade which was mainly found in the Western part of Libya among African Libyans and Western Arab Libyan. (Figure 36). The presence of the N1b1 lineage in Libya was at ~3.5% in this study which is much higher than other North African populations (Aboukhalid et al., 2013, Bekada et al., 2013, Frigi et al., 2017a, Plaza et al., 2003). This suggests that Libya received more eastern gene flow than western North Africa.

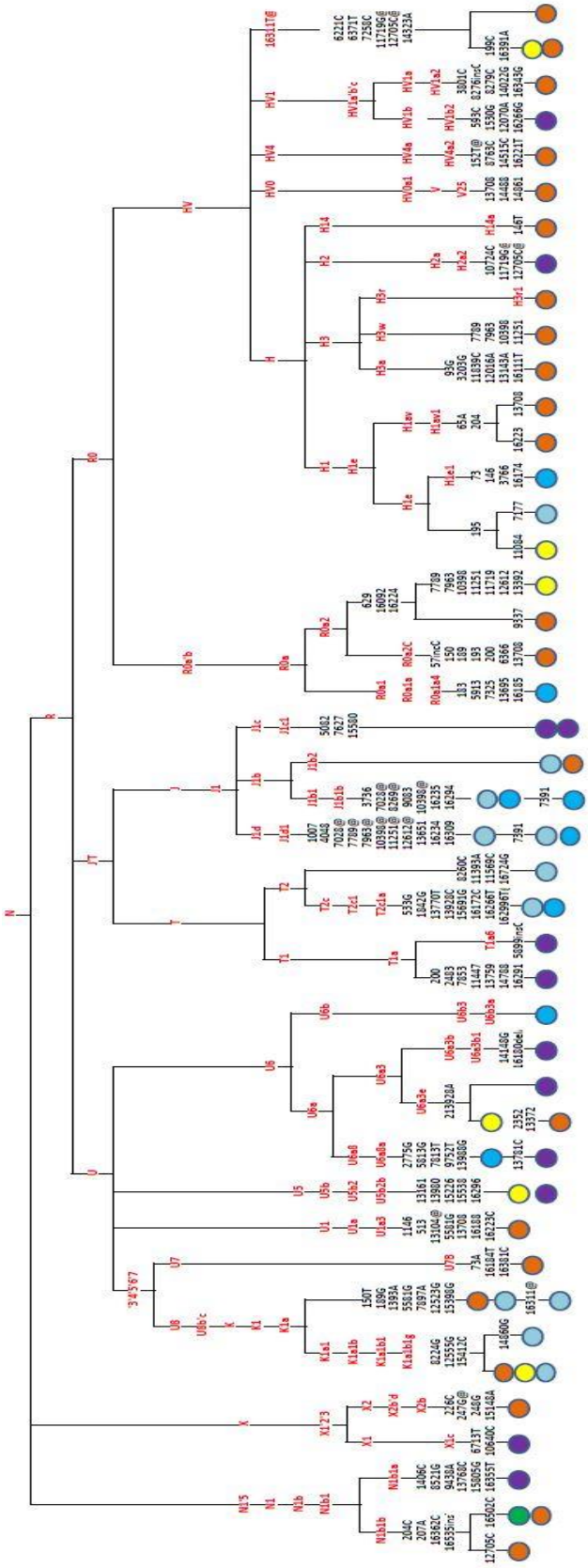

Figure 35. The tree of complete mtDNA Eurasian lineages in Libya

Western Libyan Arab samples in orange, Eastern Libyan Arab samples in, Zouara Berber in purple, Nafusa Berber in yellow, Ghadames population in blue and African Libyan in green.

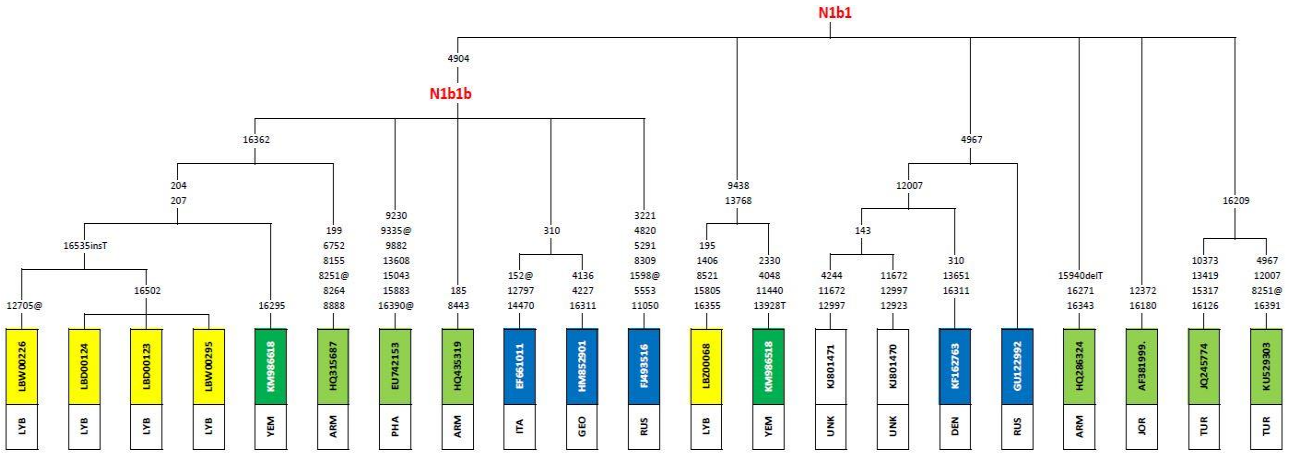

Figure 36. The tree of complete mtDNA sequences of N1b1 haplogroup

Libyan samples in yellow, Arabia in green, Near East in light green, Europe in blue and unknown samples in white.

(LBY, Libya; YEM, Yemen; JOR, Jordan; PAL, Palestine; TUR, Turkey; ARM, Armenia; ITA, Italy; RUS, Russia; DEN, Denmark; GEO, Georgia).

Macrohaplogroup R a ubiquitous outside the African continent and is the major branch of haplogroup N that evolved between Southwest Asia and Australasia (Fernandes et al., 2012). It is defined by transitions at nps 12705 and 16223 and quickly diverged once the population left Africa at nearly the same time as macrohaplogroup M and N ~60-68 Kya (Torroni et al., 2006).

This lineage represents the majority of Libyan populations in this study (69%) and reaches, 80% in the Zuwaran Berber population. The major R haplogroups found in this study include J and T, R0 including (R0a, HV) and U includes, K. HV haplogroup also including V and H

4.3.2. Super-haplogroup R0

The Libyan gene pool contains almost the whole spectrum of haplogroups deriving from the basal root of the super-haplogroup R0 (R0a, HV0, HV1, HV14a, V and H) (Figure 37).

4.1.2.1. Haplogroup (R0a)

Haplogroup (preHV) which was more recently named R0a, is the most frequent and diverse haplogroup in the Arabian Peninsula (Fernandes et al., 2012) with the highest frequency found in Socotra (Yemen) at 38% (Černý et al., 2009). The vast majority of R0a mitochondrial cluster

within R0a1 and R0a2'3 dates to the last glacial maximum (~26 kya and 21 kya, respectively), each mainly represented by a single star-like subclade, R0a1a, and R0a2. These subclades both coalesce to the Late Glacial: ~13 and 17 ka Figure 37, (Gandini et al., 2016). The age obtained for R0a haplogroup by (Černý et al., 2009) is 22.5 kya. Analysis of all R0a1 defined by variant 16355 in Yemen shows time to a most recent common ancestor (TMRCA) of 13.4 ±4.2 kya (Černý et al., 2009). Furthermore, clades R0a1a1 that is defined by variants 16172 and 16355 np identified by Abu-Amero et al. (2007) has a very young TMRCA of 5.4 kya. The basic motifs of R0a in Libya are represented by several clades; R0a1a4 which is aged between 2.6 kya and 10.7 kya years by (Behar et al., 2012b), and between 4.16 kya and 10.90 kya by (Gandini et al., 2016).

The R0a1a4 branch which is dated in this study to ~5.6 kya was only seen in Ghadames and represents around 5 % of the population. Libyan samples also shared a branch with Yemeni samples defined by 16185 dated to ~3.2 kya (Figure 37). The presence of this clade among the Ghadames population indicates multiple ancestors of this population including the Arabs.

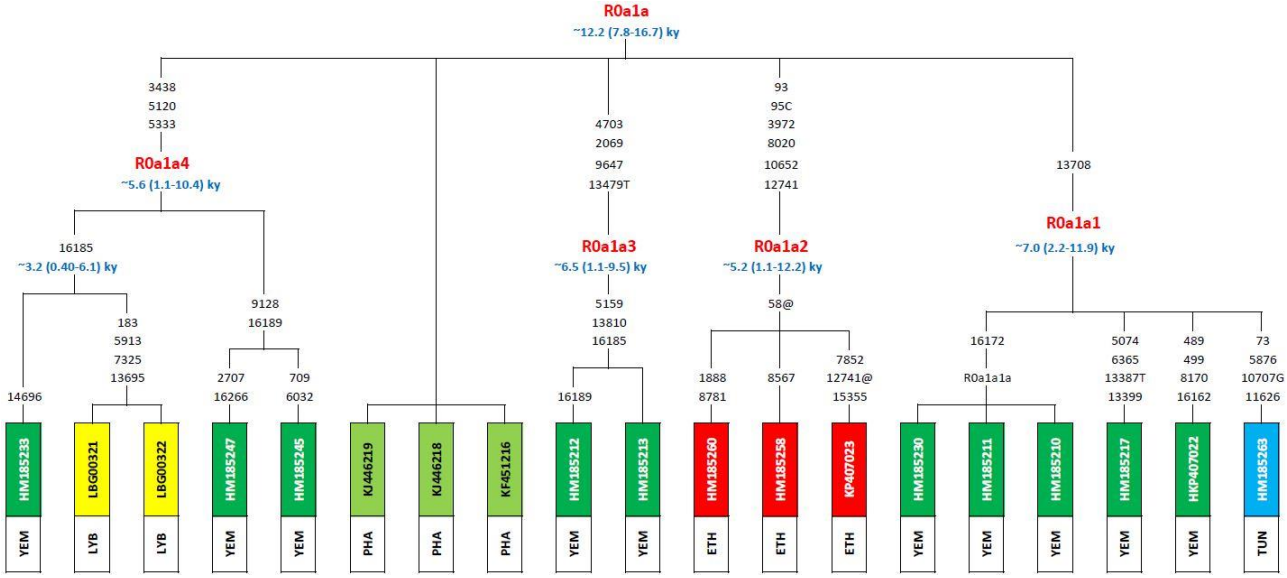

Figure 37. Tree of complete mtDNA sequences of Roa1a haplogroup

Libyan Ghadames samples in yellow, Non-Libyan North African in sky blue, sub-Saharan Africa in red, Arabia in green, Near East in light green. (LBY, Libya; IRA, Iran; YEM, Yemen; PAL, Palestine; TUN, Tunisia; ETH, Ethiopia).

Another clade found in Libya is R0a2 dated by Gandini et al. to 16.5 kya, and the date obtained in this study is 17.5 kya. R0a2 is represented by R0a2c and a sister branch R0a2i dated to 9.2 kya and 4.3 kya respectively (Gandini et al., 2016). R0a2c lineages are mainly found in Libyan Arabs at ~5 % among them. These Libyan samples belonging to R0a2c shared the branch defined by transition at 5981 with other Arabian samples dated in this study to 3.6 kya. The remaining three samples were as classified as R0a2+629+16224 branch dated in this study to 9.7 kya suggesting a Holocene expansion to Libya.

Although the entire R0a tree puts all Libyan population with Arabian Peninsula population as expected, three Libyan samples were located in a different branch of the tree Figure 38. The presence of these lineages may further confirm the gene flow from the Arabian Peninsula in the Neolithic time.

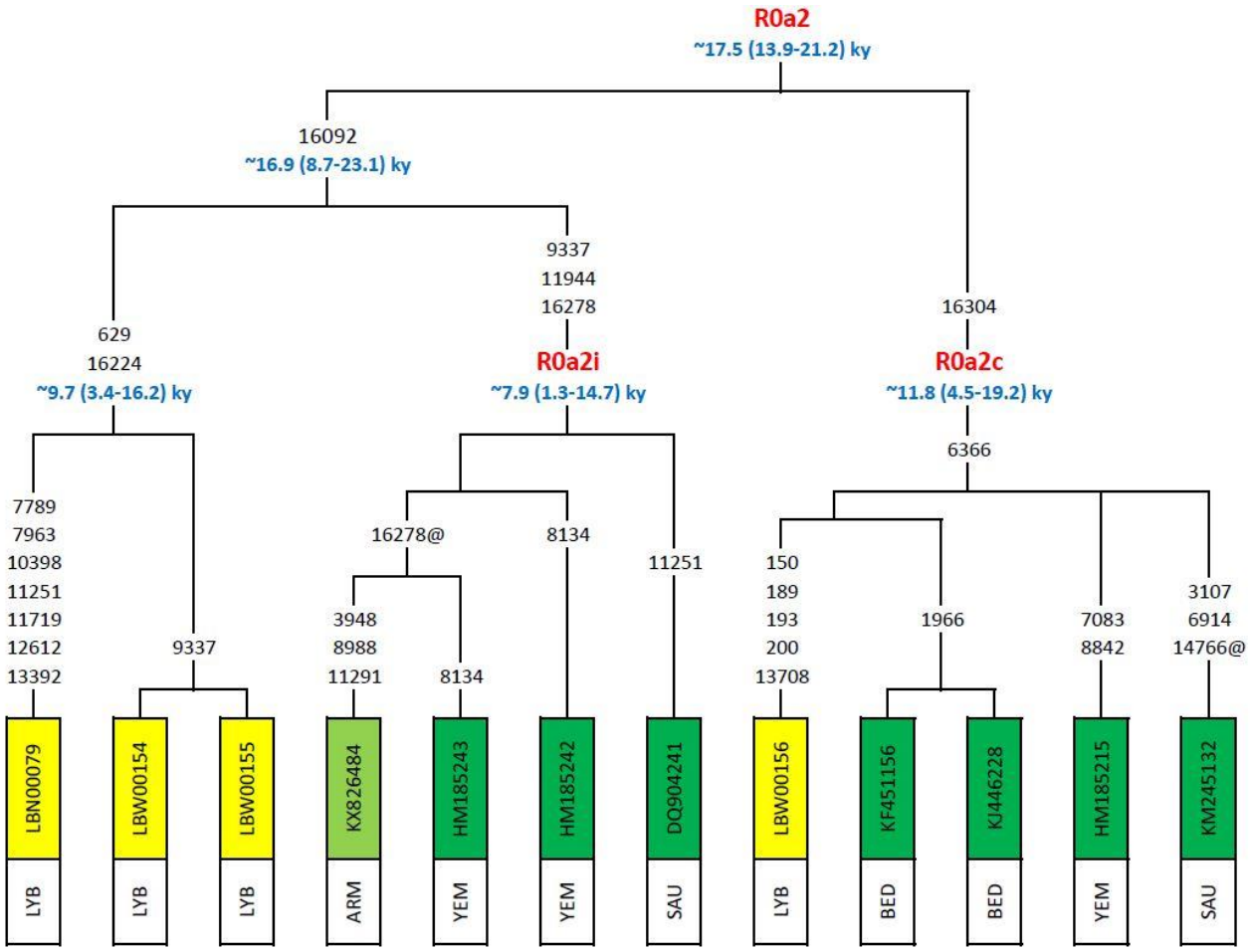

Figure 38.Tree of complete mtDNA sequences of R0a2 clade

Libyan samples in yellow, Arabia in green and Near East in light green.
(LBY, Libya; YEM, Yemen; PAL, Palestine; Arm, Armenia; SAU, Saudi; BED, Bedouin).

A founder analysis is a test using mtDNA data to identify the possible founder sequences that are shared between two populations that is considered as evidence for gene flow between the two populations. It also estimates the time of arrival of the population to the region. Gandini and colleagues performed the founder analysis between several populations to identify a possible dispersal episodes source of haplogroup R0a shown in Figure 39 (Gandini et al., 2016). They concluded that the deepest R0a branches were found in Arabia, highlighting the possible role of the Red Sea plains refugium at Pleistocene glacial, when the lineage dispersed into Eastern Africa at the end of the Late Glacial. They also suggested a Late Glacial/early postglacial dispersal into Europe through the Levantine refugium (Gandini et al., 2016).

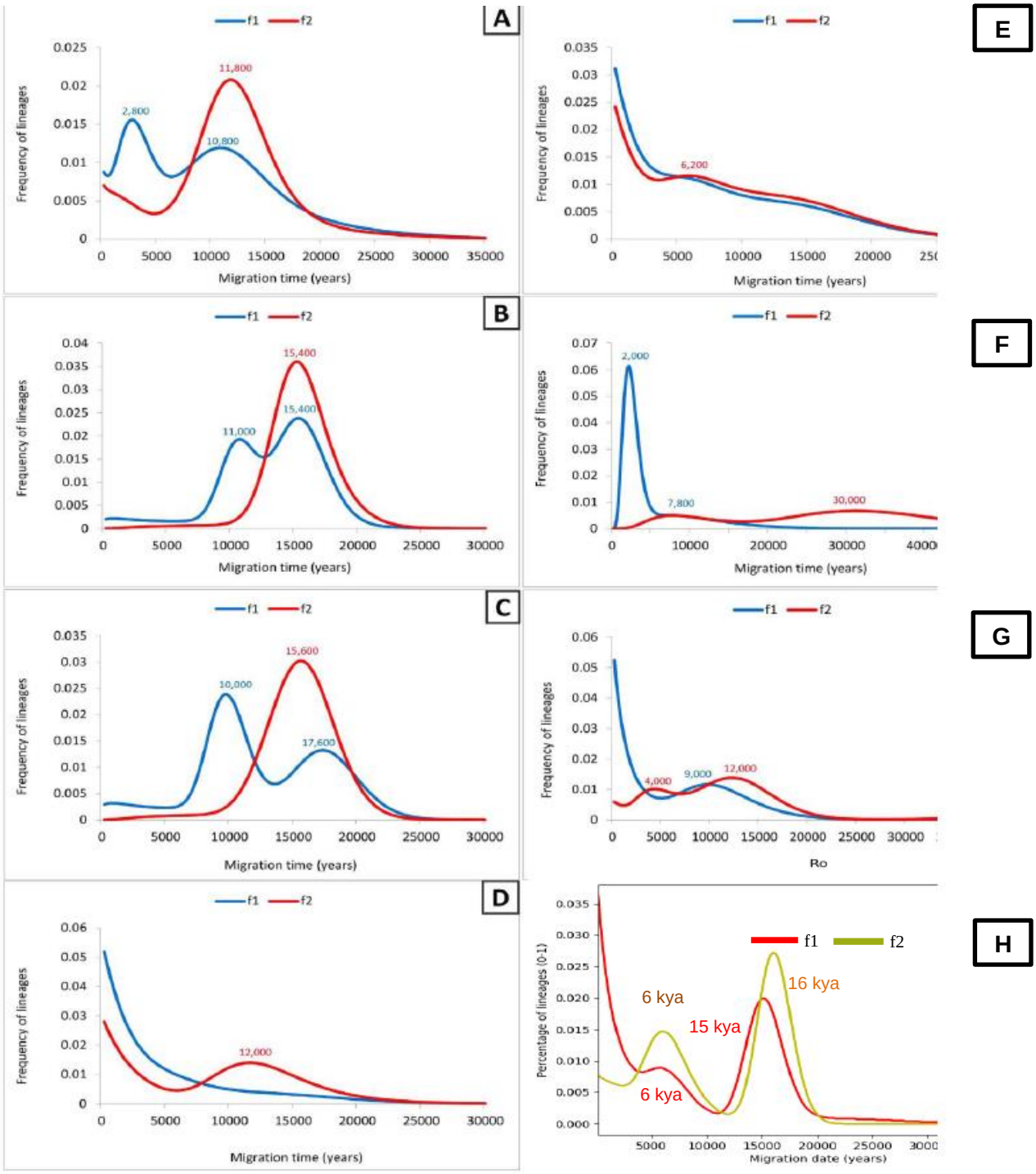

Figure 39. The probability of founder distribution of R0a lineages across migration times

(A) Migration from the Arabian Peninsula, Caucasus, Fertile Crescent and Iran to East Africa; (B) Migration from Caucasus and the Levant to Eastern Africa and Arabia; (C) Migration from Caucasus and the Fertile Crescent to the Arabian Peninsula; (D) Migration from the Arabia to Iran, Fertile Crescent and Caucasus; (E) Migration from the Arabia to the Levant and Caucasus; (F) Migration from the Iran, Fertile Crescent, the Arabia, North Africa and Caucasus to Pakistan and India (G) Migration from the Levant, Iran, Caucasus, North Africa and the Arabia to Europe. Taken from Gandini et al.,(2006) (H) Migration from the Levant, Caucasus, Iran and Arabia to North Africa done in this study. The time was scanned at each 200-year intervals from 0 to 40 kya, using f1 and f2.

Founder analysis was also performed in this study assuming that the Arabian Peninsula and the Levante are the sources of these lineages and North African populations are the sink (Figure 40). The result of founder analysis showed the presence of two dispersals from an Arabian source to North Africa; one at ~15 kya with *f1* and 16 kya at *f2,* at the Pleistocene/early Holocene corresponding to clade R0a2 and a signal at 6 kya for both *f1* and *f2* corresponding to clade R0a1a. The analysis was also performed with Libyan populations (this study) as sink Figure 40, and show a peak at 2.4 kya with *f2* corresponding to clades R0a1a4, R0a2i and R0a2c. *f1* also show a major peak towards the present (Figure 40). The dispersal to North Africa may be through a Levantine refugium, as Palestinian and the Bedouin samples were included in the tree. These finding confirmed a gene flow of R0a lineages between Arabia and North Africa at least since the Holocene time.

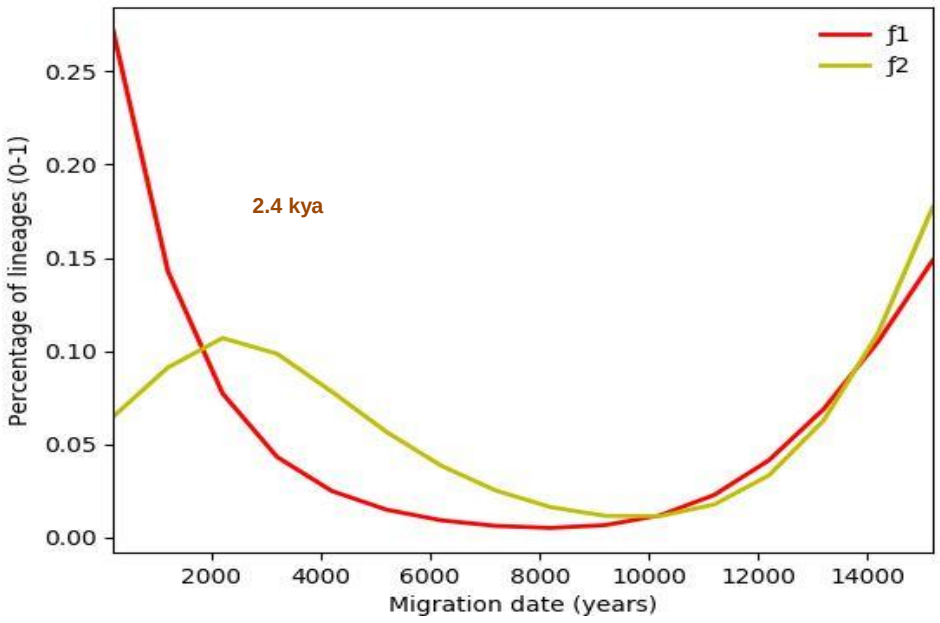

Figure 40. Founder migration of R0a lineages from the Arabian Peninsula, Fertile Crescent and Eastern Africa to Libya

4.1.2.2. Haplogroup HV

Haplogroup HV* is one of the major mitochondrial clades in haplogroup R0 and a common European pre-Neolithic origin lineage, comprising of 18 basal subclades, labelled from, HV0 to HV17 (De Fanti et al., 2015). The date of this lineage was estimated to 27 kya in the Near East and 20-23 kya in Europe (Soares et al., 2009). This lineage arose between Central and Western Asia (De Fanti et al., 2015) and is characterised by a transition at 14766. Haplogroup HV, which is the ancestral haplogroup to haplogroup H and haplogroup V (Richards et al., 2000a) is found in high frequency (19–24%) in Southern Europe (Italy and Spain) ranging in the whole of Europe around 10%. HV represents 7.85% of the Libyan population. Four of the Libyan samples in this study were classified as HV* at the branch defined by the mutation T16311C! (previously designated as HV3) and includes HV6-HV11 and HV14-HV17 (Figure 41). The 16311 branches dated to 11.2 kya (De Fanti et al., 2015), while it was found to be 10 kya in the study with the Libyan samples dated to 4 kya.

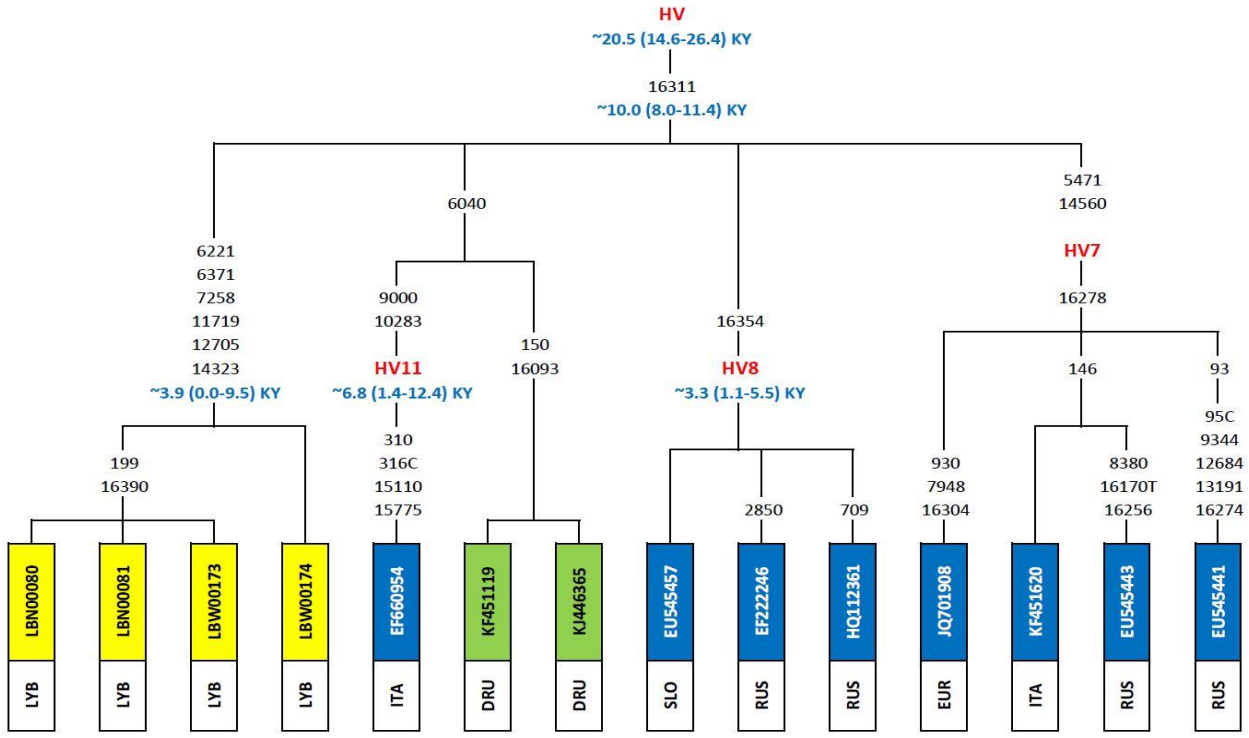

Figure 41. Tree of complete mtDNA sequences of HV+16311 lineages

Libyan samples in yellow, Near East in light green and Europe in blue (LBY, Libya; ITA, Italy; DRU, Druz; SLO, Slovenia; RUS, Russa).

4.1.2.2.1. Haplogroup HV0

HV0 is a sister clade of H, defined by transitions at 16298 and 72 which originated in Europe before the LGM, 17 kya (De Fanti et al., 2015). HV0 accounts for 5% of European mtDNAs and tends to be exclusive to western, central, and northern Europe with higher frequencies in northern Italy, although it is uncommon in the Mediterranean region (Torroni et al., 2001), HV0 mtDNAs were observed in three subjects belonging to the western part of Libya. Nafusa sample was located in a basal branch with European samples; other Libyan samples were located along with other North African samples. The Libyan samples in this clade HV showed proximity to other North African samples including Egypt Figure 42. Although this haplogroup is European in origin, the low diversity in this lineage in North Africa probably indicates of recent migration in the region rather than post-last glacial maximum expansion from south-west Europe.

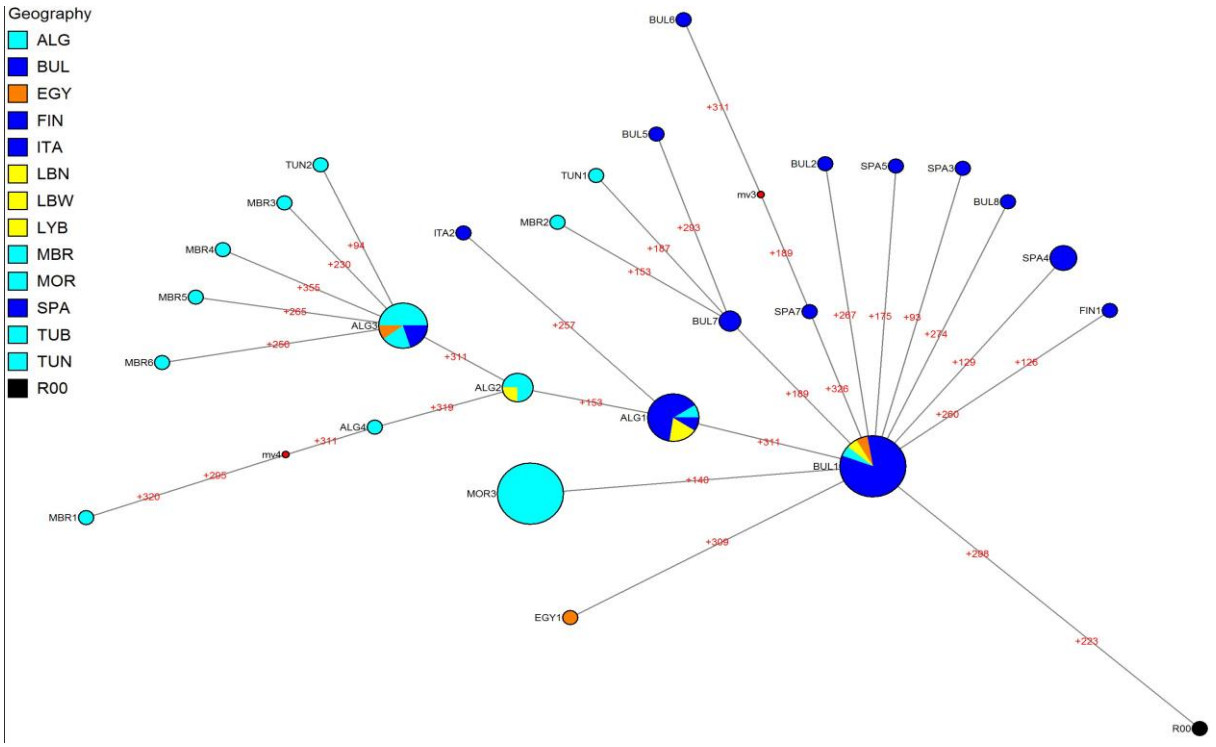

Figure 42. The HVS-I Reduced-median network for haplogroup HV0

Libyan samples in yellow, North African samples in sky blue, Egyptian in orange and European samples in dark blue (LBN, Nafusa Berber; LBW, Libyan western Arab; LYB, General Libyan; SPA, Spain; MBR, MOR, Morocco; ALG, Algeria;; FIN, Finland; BUL, Bulgaria; EGY, Egypt; ITA, Italy; TUN, TUB, Tunisia)

4.1.2.2.1.1. Sub-clade V

Sub-clade V is autochthonous European sub-clade arisen from haplogroup HV0 defined by G4580A, and the absence of the 4577 that arose in the Late Glacial period, sometime between 16 kya and 12 kya in northern Iberia or southwestern France at about the time of the Younger Dryas (Gómez-Carballa et al., 2012). Haplogroup V is one of the frequent in clades found in the Basques (Barral-Arca et al., 2016).

Two West Libyan Arab samples were in branch V25 dated in this study to 10.5 kya. This V25 samples share a clade with North African Berber sequences from Algeria and Morocco. The branch is defined by a mutation at 14629 and was dated in this study to 6.6 kya Figure 43.

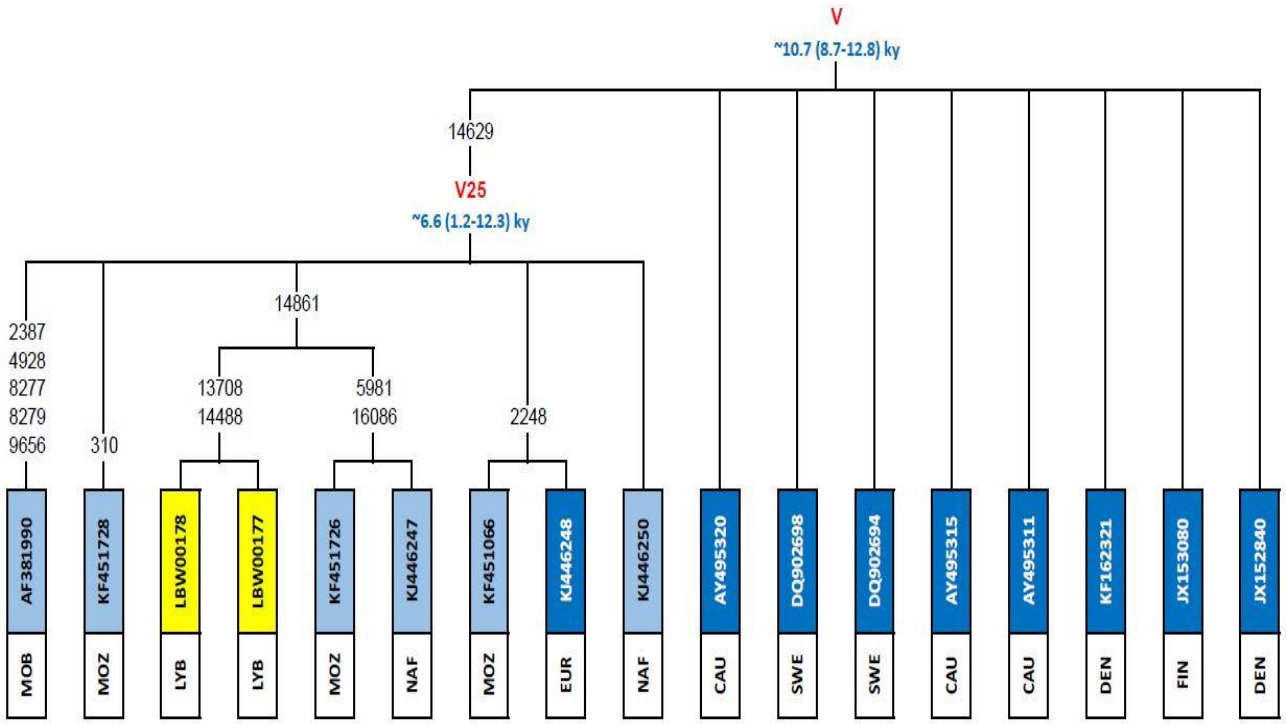

Figure 43. Tree of complete mtDNA sequences of V lineage

Libyan samples in yellow, North African in sky blue and Europe in blue (DEN, Denmark; LYB, Libya; MOB, Moroccan Berber; MOZ, Algeria Berber; NAF, North Africa; CAU, Caucuses; SWE, Sweden; FIN, Finland; EUR, Europe)

4.1.2.2.3. HV1 haplogroup

The HV1 lineage is defined by 16067 and 16343 transitions and is found in Arabia, Eastern and Northern Africa and attains a frequency of 15.7% in Yemen (Černý et al., 2009), 18.4% in Saudi Arabia (Abu-Amero et al., 2008), 4.9% in Sudan, 11.2% in Ethiopia, and 9.5% in Somalia (Figure 44) (Musilová et al., 2011). Little information about this branch of HV1 haplogroup, which is dated between 20 and 24 kya in the Near East by (Musilová et al., 2011), overlapping the LGM period (14,737–30,227) by (Torroni et al., 2001) and 21 kya in this study. Libyan samples in this clade are located in branch defined by a transversion (A to T) at 8,014 and two transitions at positions 15,218 and 16,067 (van Oven and Kayser, 2009) (Figure 45, 46).

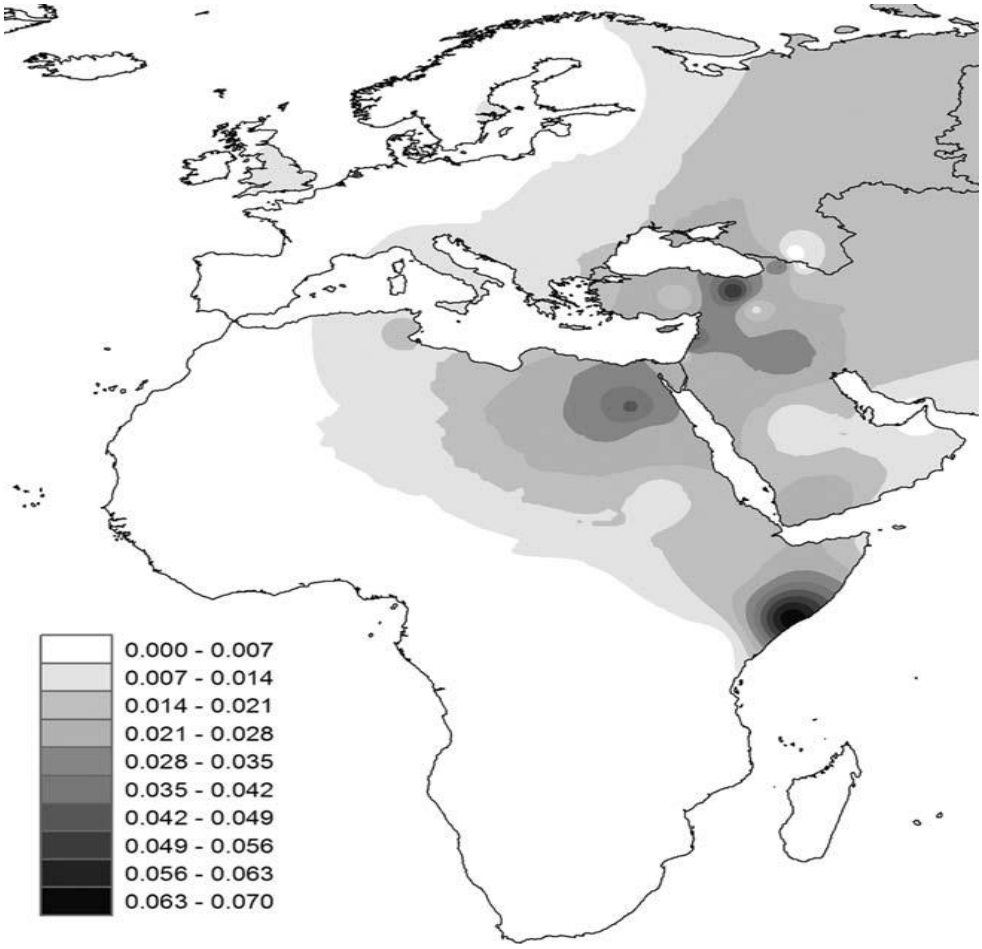

Figure 44. Interpolation map for HV1 haplogroup

from Musilova et al., 2011

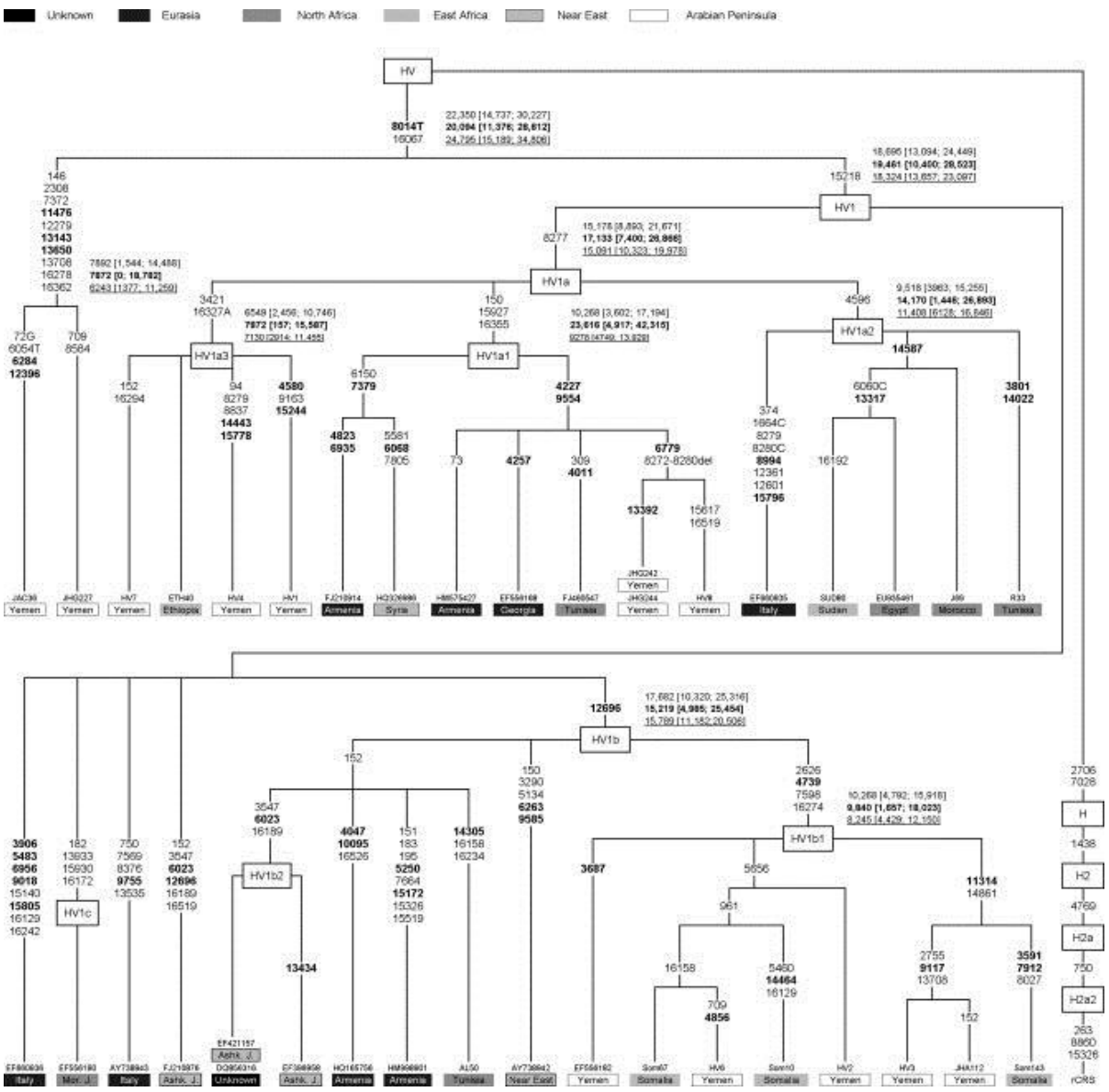

Figure 45. Phylogeny of the complete HV1 mtDNA sequences

Taken from (Musilová et al., 2011)

Two HV1 lineages were found in Libyan samples in this study, HV1a2 and, HV1b2. HV1a2 clade is defined by a transition at 4596, dated to 9.5 kya by (Musilová et al., 2011), and in this study was found at 8.5 kya. This clade is mainly found in Arabia and North African samples. The HV1a2 Libyan samples were located with a Tunisian sample in the same branch defined by transition at 3801 and 14022. This branch was dated in this study to 2.5 kya, Figure 46.

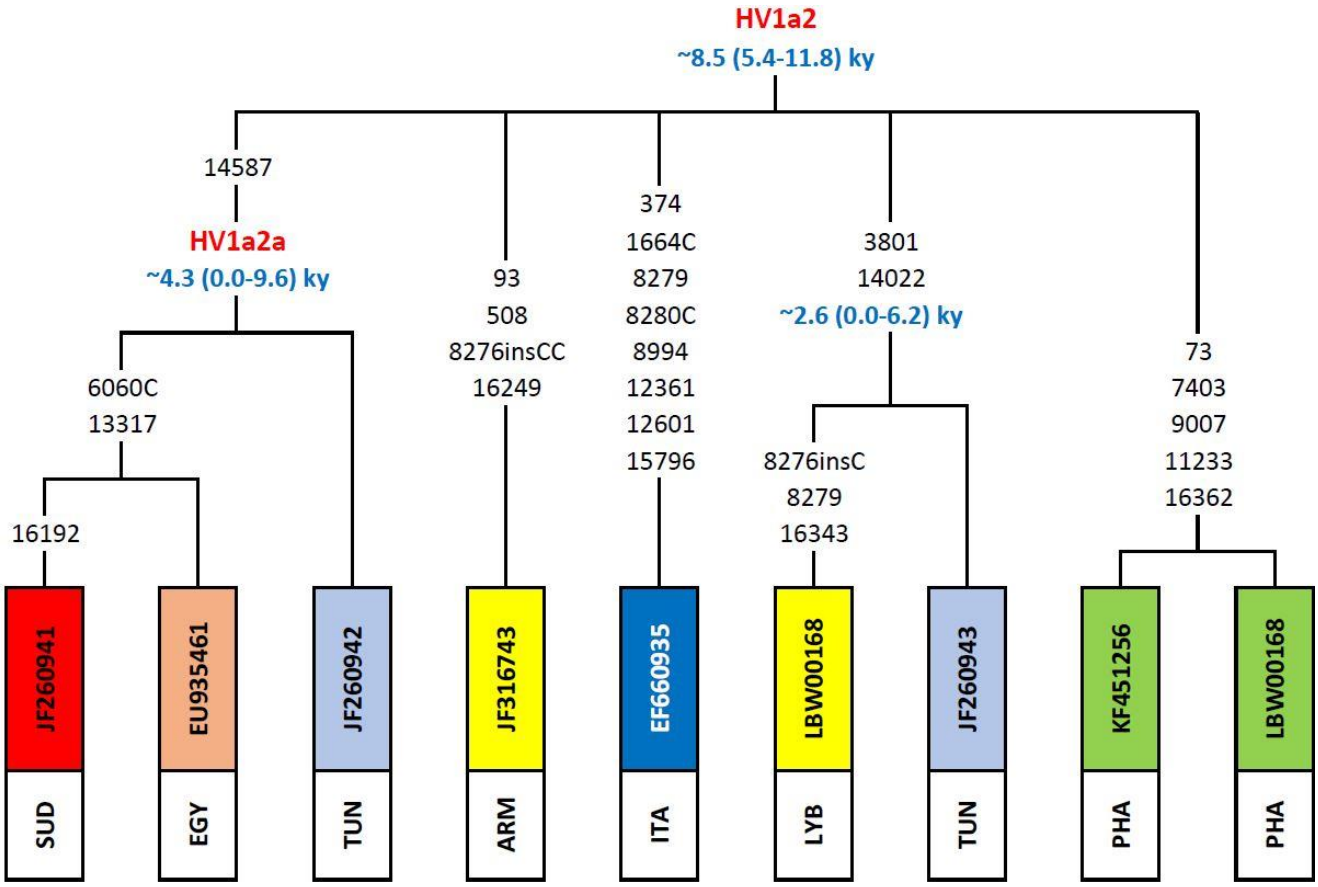

Figure 46. The tree of mtDNA complete sequences of HV1a2 lineage

Libyan Western Arab samples in yellow, North African in sky blue, sub-Saharan Africa in red, Egypt in orange, Near East in light green, and Europe in blue (LYB, Libya; EGY; Egypt; ITA, Italy; ARM, Armenia; SUD, Sudan; TUN, Tunisia; PHA, Palestine)

Another HV1 clade found among Libyan samples in this study is a Near Eastern sub-haplogroup HV1b2, a branch of HV1b that presents substitutions at positions 152, 3,547, 6,023, and 16,189, dated to 2 kya (Costa et al., 2013b). This clade was found among the Libyan Berber Zuwara, and is found among Ashkenazi Jews (Figure 47), and dated in this study to 7.6 kya (Figure 48). The presence of this in the Zuwara population strengthens the picture obtained from the position of Zuwara population in PCA that showed the proximity of Zuwara with the Levant population and suggests an early Levantine founder for some of the Zuwaran population.

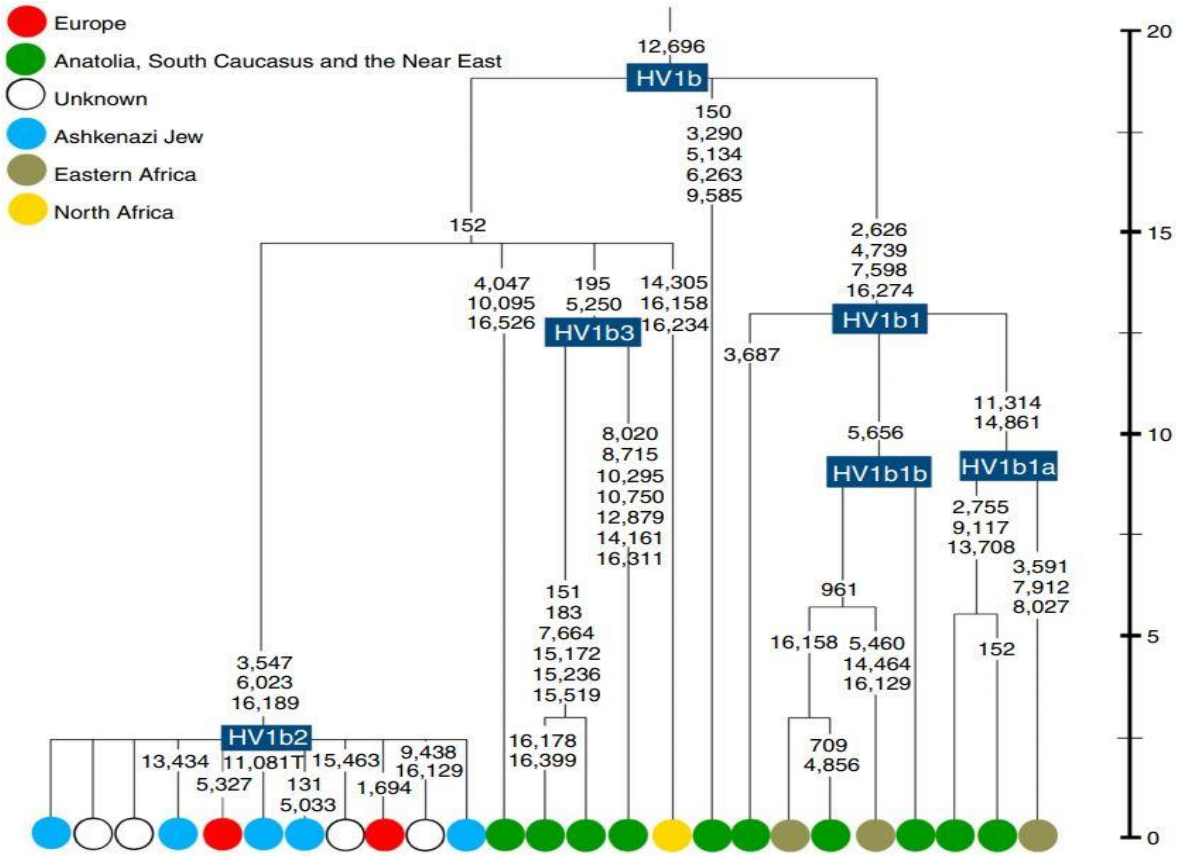

Figure 47. Phylogenetic tree of haplogroup HV1b
Adapted from (Costa et al., 2013b)

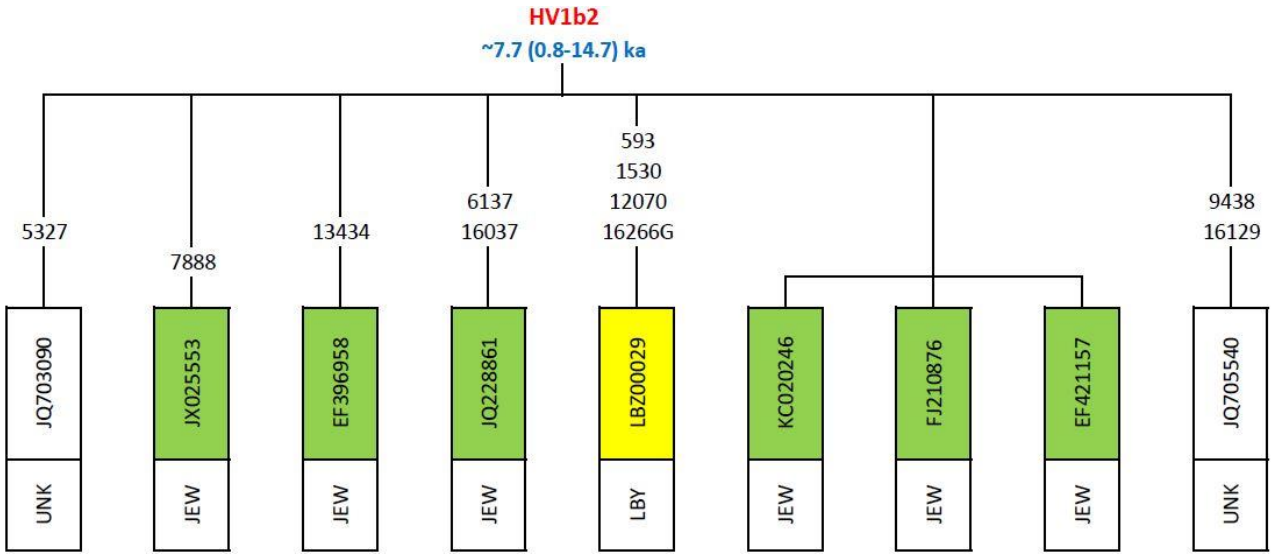

Figure 48. Tree of complete mtDNA sequences of HV1b2 lineage.

Libyan samples in yellow, Near East in light green and unknown samples in white LBY, Libya; JEW, Ashkenazi Jewish; UNK, Unknown)

4.1.2.2.4. Haplogroup HV4

HV4 is a monophyletic lineage characterised by the mutation at T7094C that is found in different locations in Central-Southern Italy (De Fanti et al., 2015). However, Gómez-Carballa and his colleagues found that HV4 originated in Eastern Europe 14.2 kya (Gómez-Carballa et al., 2012) between 11.4 kya and 20.3 kya (Behar et al., 2012b). HV4a was dated to ~15 kya (De Fanti et al., 2015) and 15.6 kya in this study.

Clade HV4a2 is defined by transitions at 7805 and 16129 and is the earliest splitting clade among HV14a (De Fanti et al., 2015) originating in the Middle East around 9.3 kya. However, some diffusions into the Mediterranean also considered as this clade are seen in Italy (Figure 49, 50) (Gómez-Carballa et al., 2012).

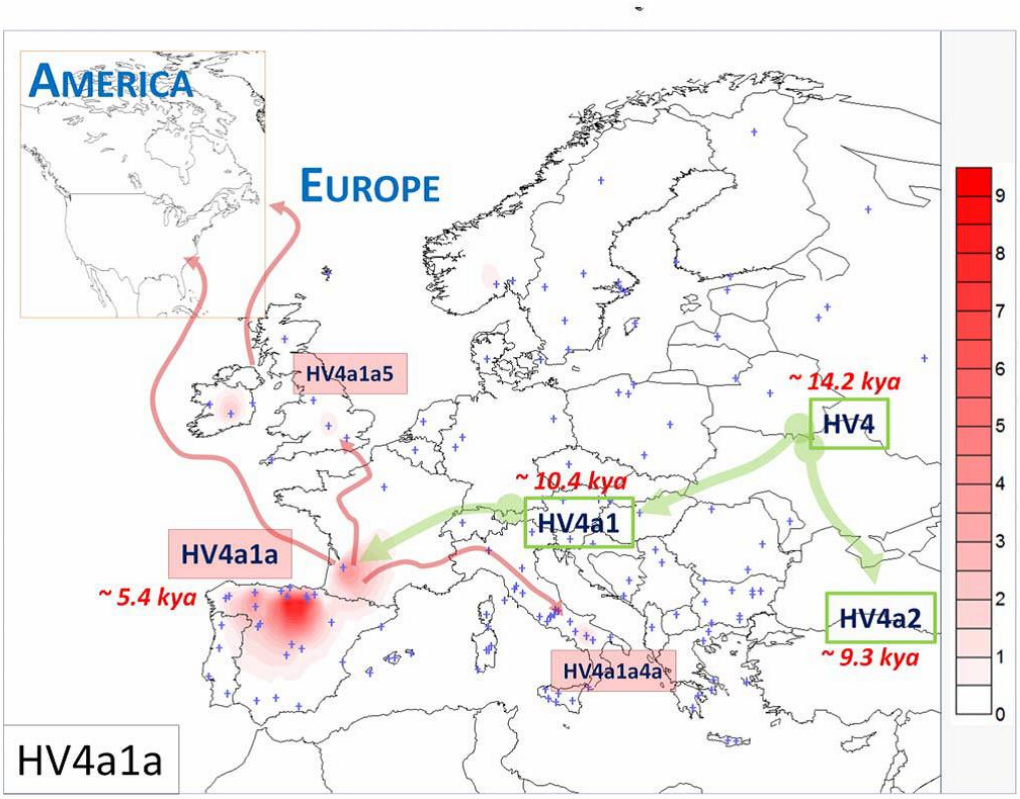

Figure 49. Map of Europe showing the frequency distribution of haplogroup HV4a
Adapted from (Gómez-Carballa et al., 2012)

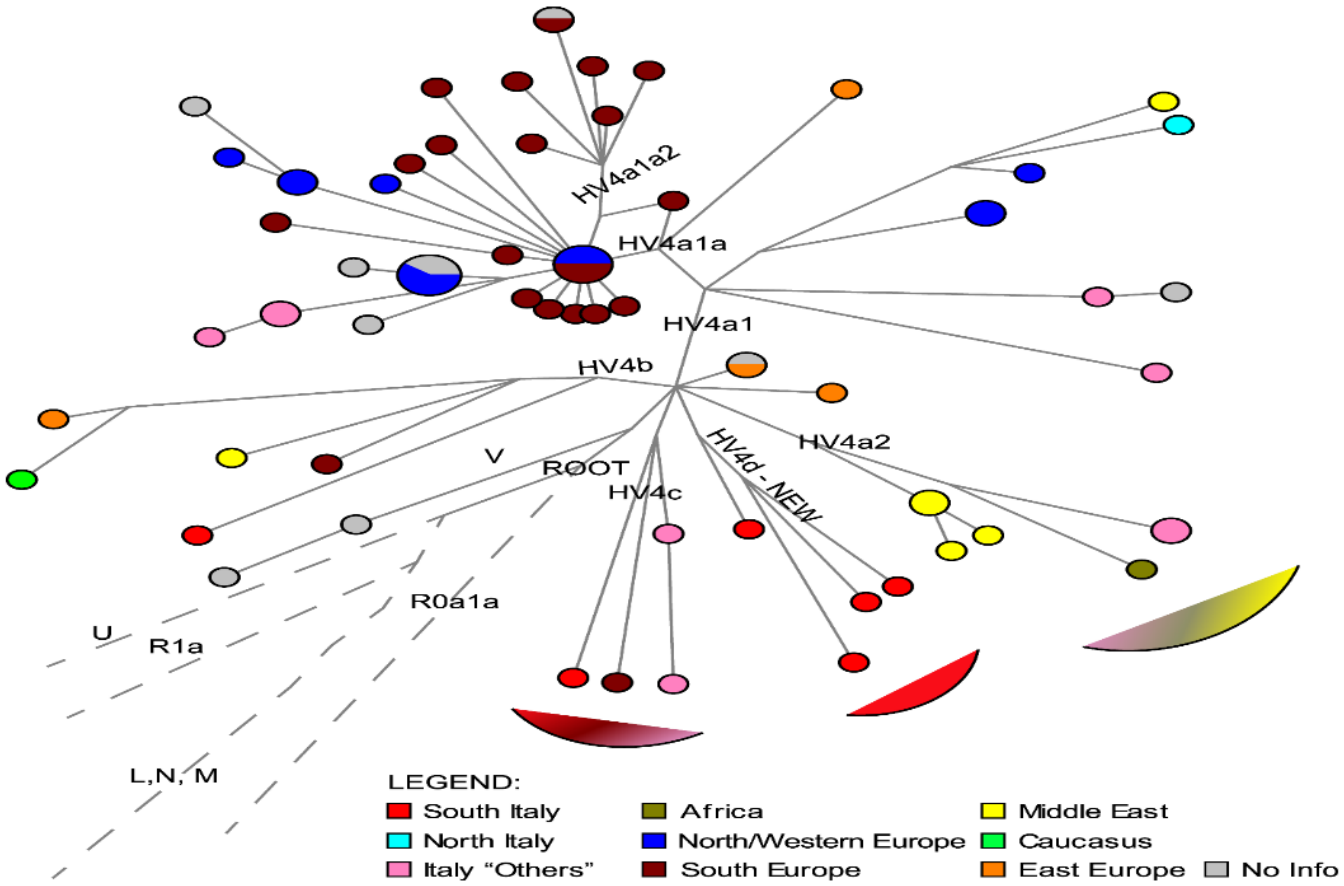

Figure 50. Tree of complete mtDNA sequences belonging to haplogroup HV4

Taken from (Gómez-Carballa et al., 2012)

All samples represented in this clade in Libya are samples found in western Libya belonging to the Arabic group, these fall into a branch defined by transition 16221, which also includes one Jordanian and three Assyrian Iraqi samples Figures 51, 52. In this study, HV4a2 has estimated to 11.1 kya, and the age of clade contains Libyan samples estimated to 1.5 kya (Figure 52). Again, as the tree include samples from Jordan and Iraq within the same clade with Libyan samples. This may be another a sign of the influence of Levant and Arabia in Libya.

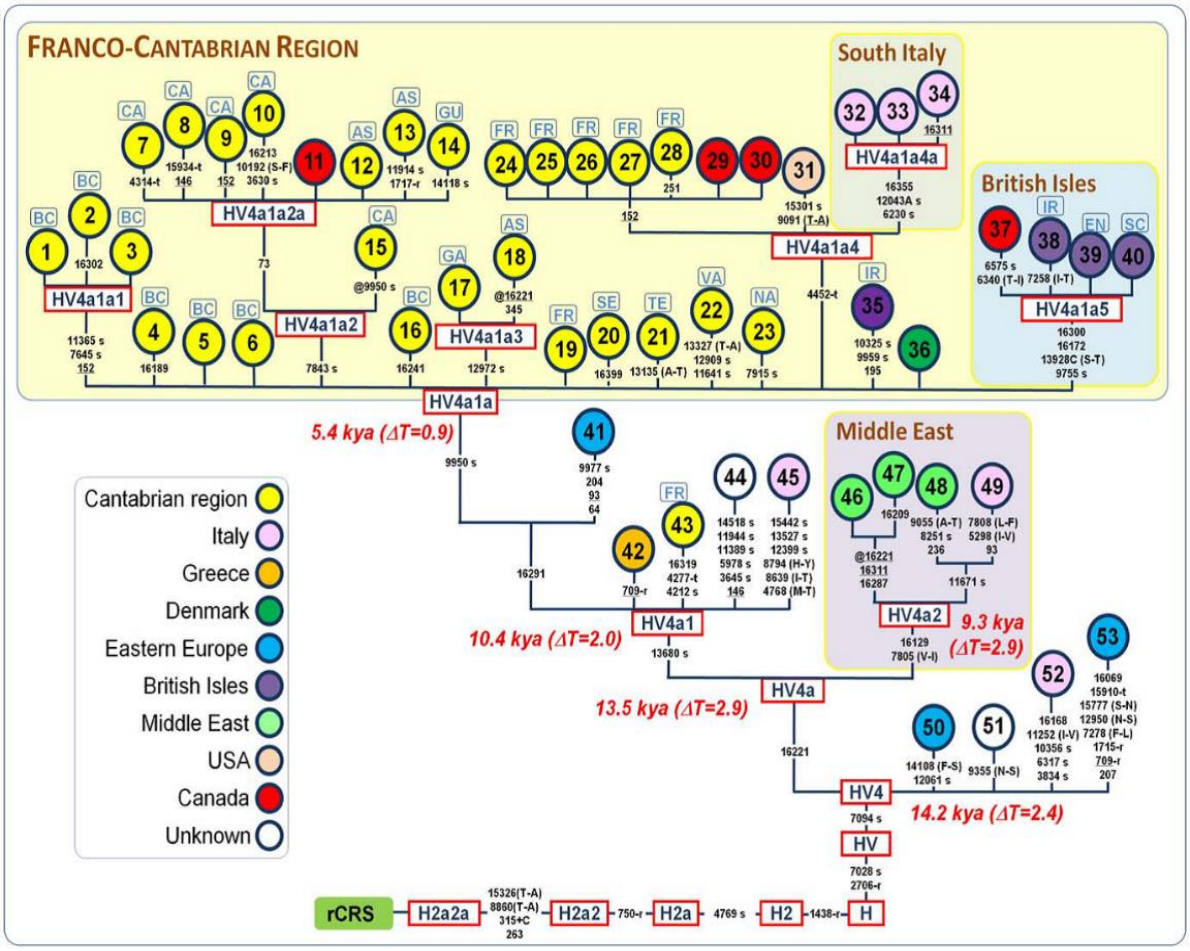

Figure 51. Maximum parsimony tree of HV4 haplogroup using complete mtDNA genomes

Adopted from (Gómez-Carballa et al., 2012)

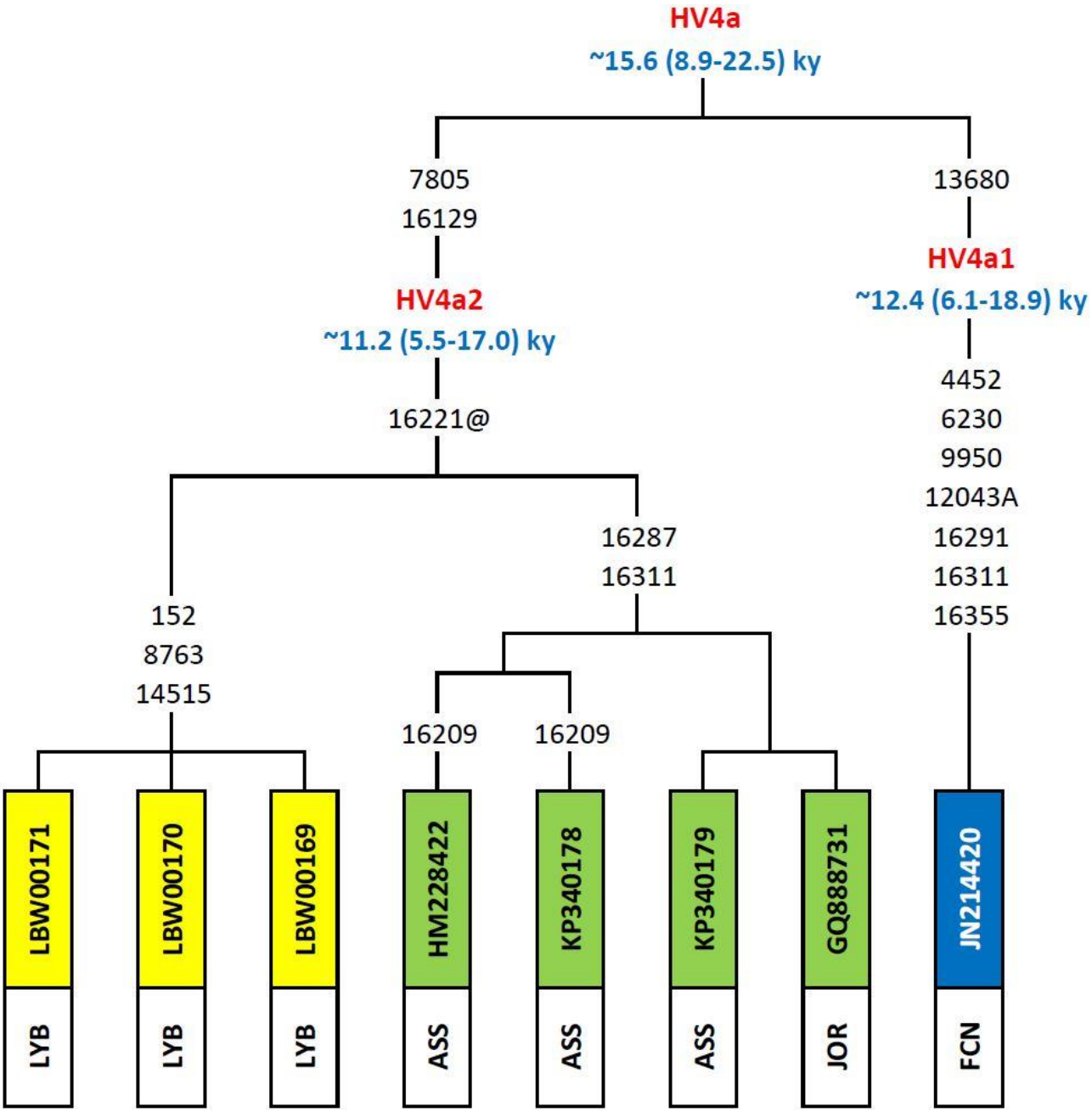

Figure 52. The tree of mtDNA complete sequences of HV4a lineage

Libyan western Arab samples in yellow, the Middle East in light green, and Europe in blue (LYB, Libya; ASS, Assyrian; JOR, Jordan FCN, Franco Cantabria)

Founder analysis was also performed in this study assuming that the Arabian Peninsula, the Levant and Europe are the sources of this lineages and Libyan populations are the sink (Figure 53). The result of founder analysis showed the presence of two dispersals into Libya. The major peak is around 8.2 kya, that may corresponding to haplogroups HV0 and HV*. Similar finding were also

stated by Torroni where they found strong evidence for European genetic input into North Africa such as haplogroup V (Torroni et al., 1998), that arrived either later Neolithic time (Rando et al., 1998). The other peak at 19.8 kya may refer to haplogroup HV* that dispersed to North Africa may through a Levantine refugium.

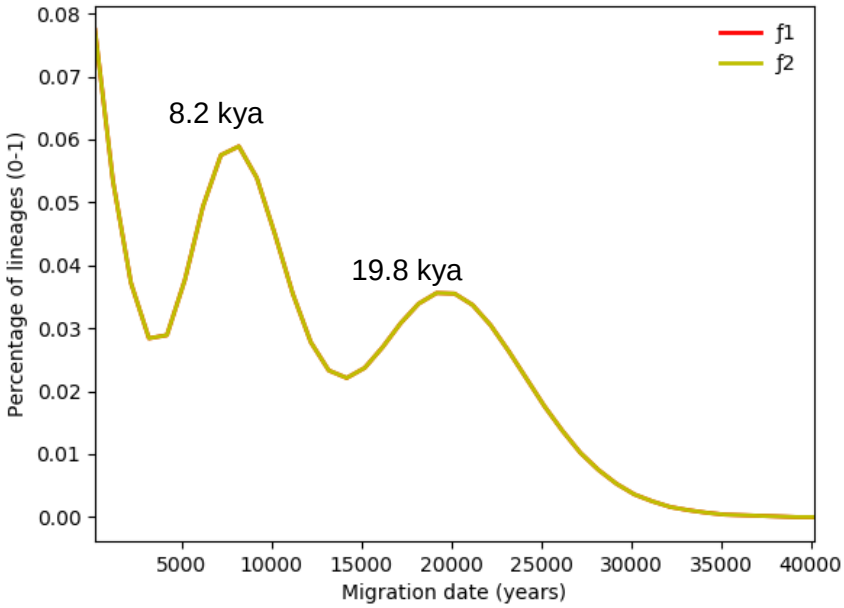

Figure 53. Founder distribution of HV lineages across migration times

The analysis of HV among Libyan samples may indicate the presence of different origin for the Libyan populations while individuals with HV0 haplogroup are more related to the European population. It is also clear the proximity of HV1 Libyan population individuals to the Eastern populations in Egypt and the Arabian Peninsula.

4.1.2.3. Haplogroup H

Haplogroup H is predominantly found in Europe and is characterized by 2706 and 7028 transitions (Hernández et al., 2017). Several types of research have suggested that haplogroup H primarily originated in the Middle East between 30–25 kya; and spread into Europe with a second Paleolithic wave. It may be accompanied with the Gravettian technology (25–20 kya); and was most likely involved in the expansions from ice-age refugia after the LGM (Torroni et al. 1998; Richards et al.

2000). Others have suggested that haplogroup H was introduced ~45–40 kya about proto colonisation or by migration in the Middle/Late Upper Paleolithic, Neolithic period (Torroni et al. 1998; Richards et al. 2000). Haplogroup H occupies more than a third (~40-45% on average) of human mitochondrial DNA gene pool in Europe (Brotherton et al., 2013) and 20%–30% in the Near East and the Caucasus region (Roostalu et al., 2007, Zsurka et al., 2007); however its much lower in Arabian Peninsula where the mean frequency is 9 %. It accounts for 13% of Saudi lineages (Abu-Amero et al., 2007a, Abu-Amero et al., 2008).

In this study, the H haplogroup is the most abundant among the general Libyan population being ~20%. The Nafusa Berber population has the highest percentage of haplogroup H 26.2% whereas Ghadames group possess the lowest percentage being 8.9% (Table 11). Libyan Tuareg also carries a high frequency of haplogroup H1 (60%), (Ottoni et al., 2010). An admixture of western and eastern H1, H2, H3, H4, H6, H7 and H14 subclades were found within haplogroup H in the current study.

4.1.2.3.1. H1 Haplogroup

Haplogroup H1 is common in Western European populations at 40%-50% and has a coalescence time of about 8–9 kya in the European (Ottoni et al., 2010). H1 also is the dominant H subgroups in North Africa at 42%, which is similar to the Iberian Peninsula. (Ennafaa et al., 2009). This suggests an arrival time of haplogroup H1 in North Africa from Iberia after the Younger Dryas cold snap at the early Holocene (Ottoni et al., 2010). This was followed by *in situ* formations of a specific North African clade of H1 with sub-haplogroups such as haplogroup H1v2 defined by 10101 C and 146 C. H1v found among Libyan Tuareg Figure 54, (Ottoni et al., 2010) was dated to 4 kya (Font-Porterias et al., 2018). Haplogroup H1 was also the abundant clade among Libyans in this study accounting 37% of H lineages (Figure 55). Haplogroup H1 was dated in this study to 11.7 kya.

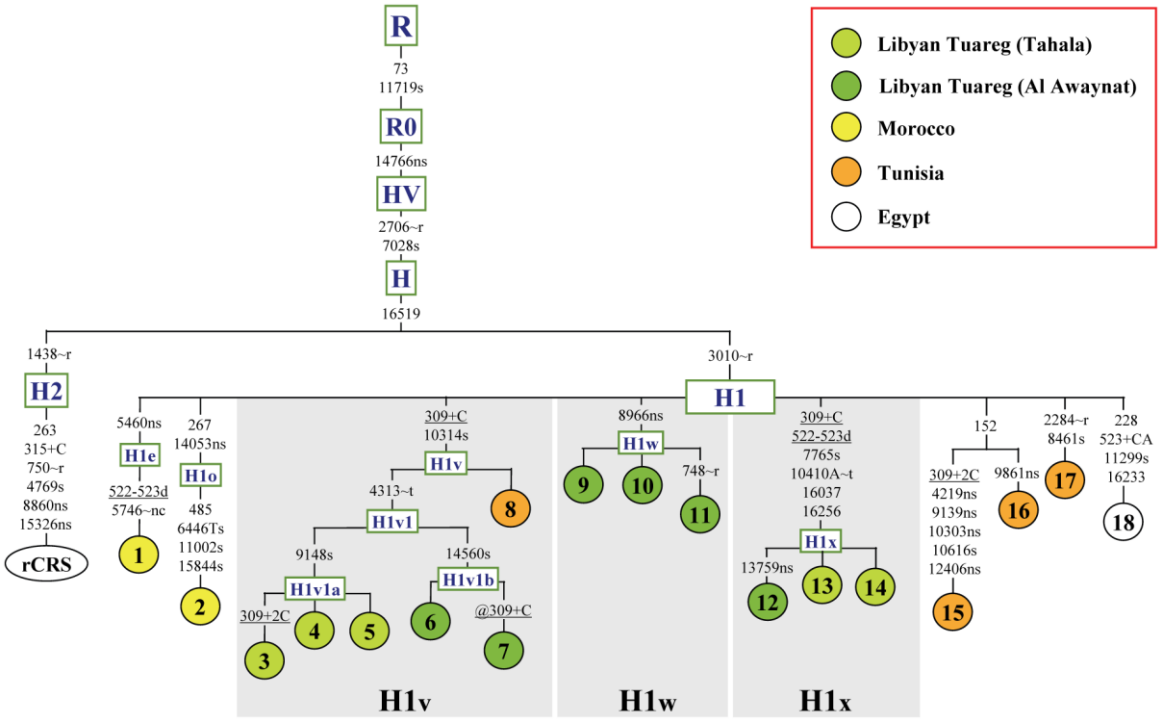

Figure 54. Tree of complete H1 mtDNA sequences in North Africa

Taken from (Ottoni et al., 2010)

4.1.2.3.2. Haplogroup H1e

Haplogroup H1e is defined by transition at np 5460 and dated to 10.8 kya in this study found in five samples, four of them were from East Libya and one from Nafusa Mountain population that lies in the branch defined by transition at 195, dated in this study to 2.6 kya and the last sample was classified as H1e1 belonging to Ghadames population and dated in this study to 8.8 kya. Interestingly, the majority of the samples found in this branch are European (Figure 55).

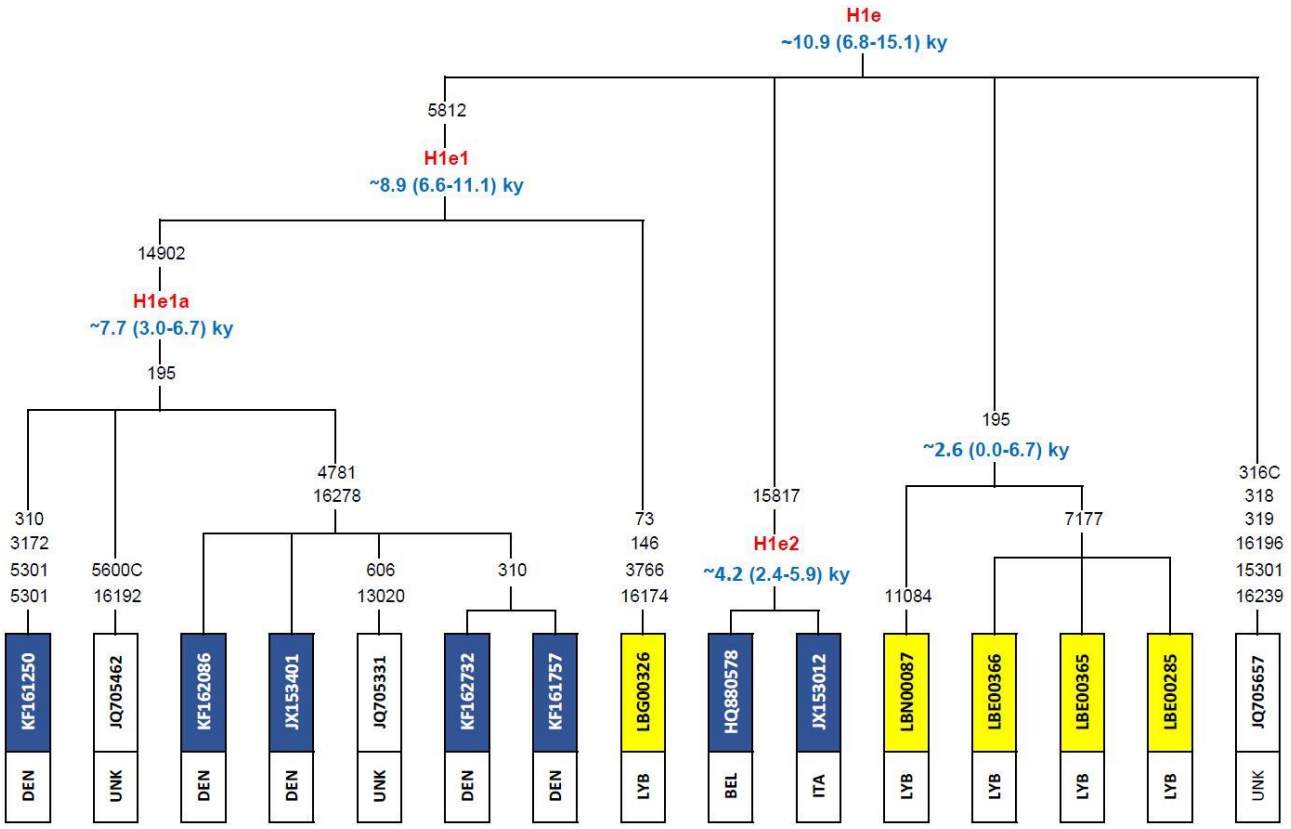

Figure 55. Tree of complete mtDNA sequences of H1e lineage

Libyan samples in yellow, and European in blue (DEN, Denmark; LYB, Libya; BEL, Belorussia; ITA, Italy; UNK, Unknown)

4.1.2.3.3. Haplogroup H1av

Haplogroup H1av is a branch of haplogroup H1 defined by G7762A transition, first found in Basque population and accounts for 4% of H haplogroup variation. H1av haplogroup distribution was initially restricted to Basque-speaking populations and spread to the surrounding Spanish populations (Behar et al., 2012a). H1av was dated in this study to 6.8 kya. The main branch of H1av, H1av1 is defined by transition at C150T dated to 3.2 kya, (Figure 56) (Behar et al., 2012a).

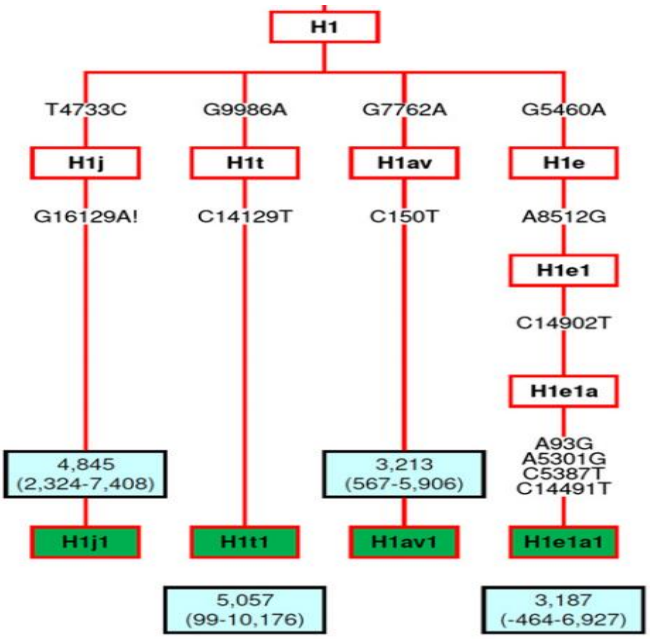

Figure 56. Phylogenetic Tree of the H1av haplogroup

Taken from (Behar et al., 2012a)

Five Western Libyan Arab subjects were found in this study belonging to the western population were located in the H1av1 branch which was dated in this study to 3.7 kya (Figure 57). The vast majority of samples in this branch are Basque or Spanish suggesting gene flow between Iberia and North Africa.

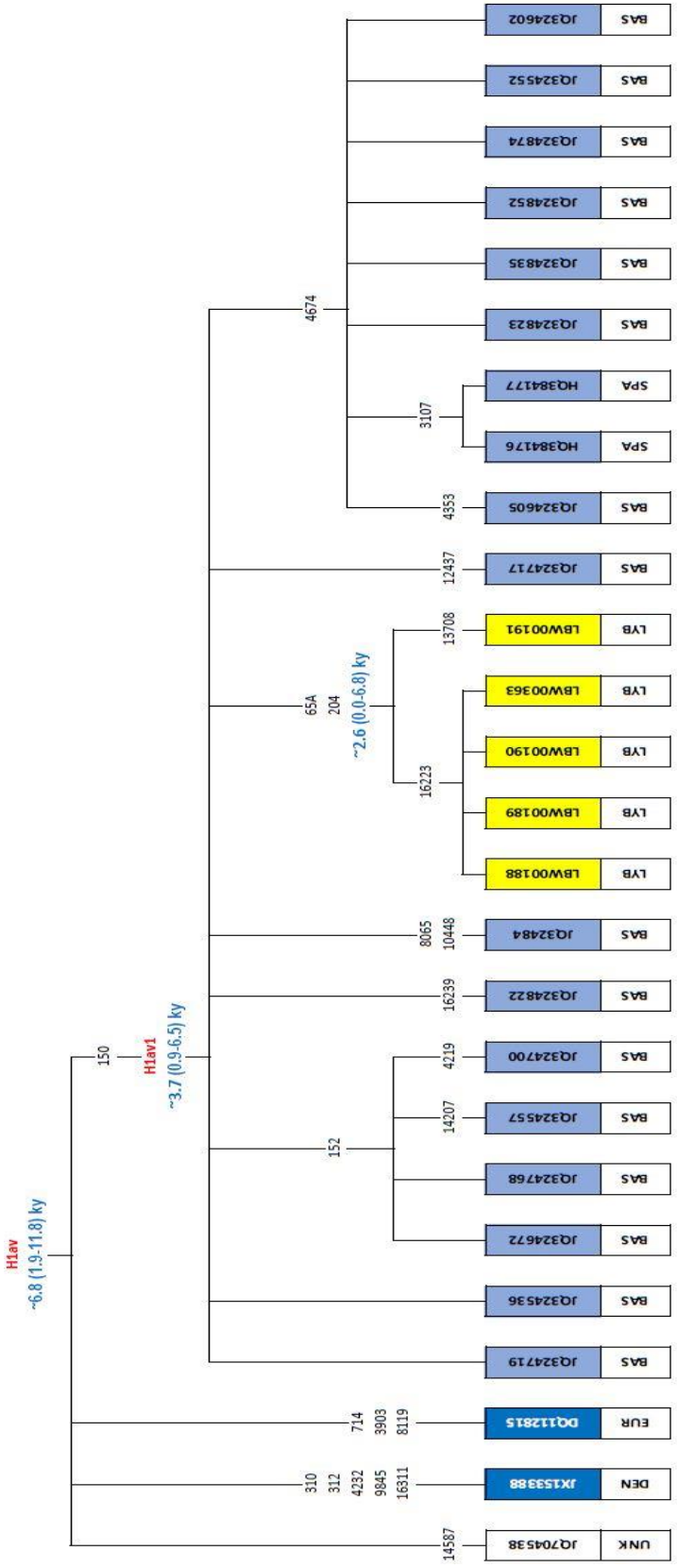

Figure 57. Tree of complete mtDNA sequences of H1av lineage

(Libyan samples in yellow, Europe in blue and light blue for Iberian samples (DEN, Denmark; LYB, Libya; BAS, Basque; SPA, Spain; UNK, Unknown)

4.1.2.3.4. Haplogroup H3

The haplogroup H3 lineage is characterised by a transition from T6776C dated to 10.3 kya in the Iberian Peninsula and found at highest frequencies in Europe in the Cantabrian Cornice and Southern Iberia (Ennafaa et al., 2009). Similar to haplogroups H1 and H3 it has coalescent ages at 11 kya in North Africa suggesting a late Palaeolithic settlement.

H3 represents 13% of Haplogroup H in North Africa and only 7% of Libyan H and shows a west-east gradient distribution of this clade in North Africa. (Ennafaa et al., 2009) Five samples belonging to different branches of H3 were found in Libyan dataset. All the samples are Arab, two of them classified as H3d. The other samples in the tree are either Iberian or southern Italy (Figure 58). Presence of this lineage in Libya is another evidence of gene flow from Iberian peninsula through the Strait of Gibraltar at the end of the LGM.

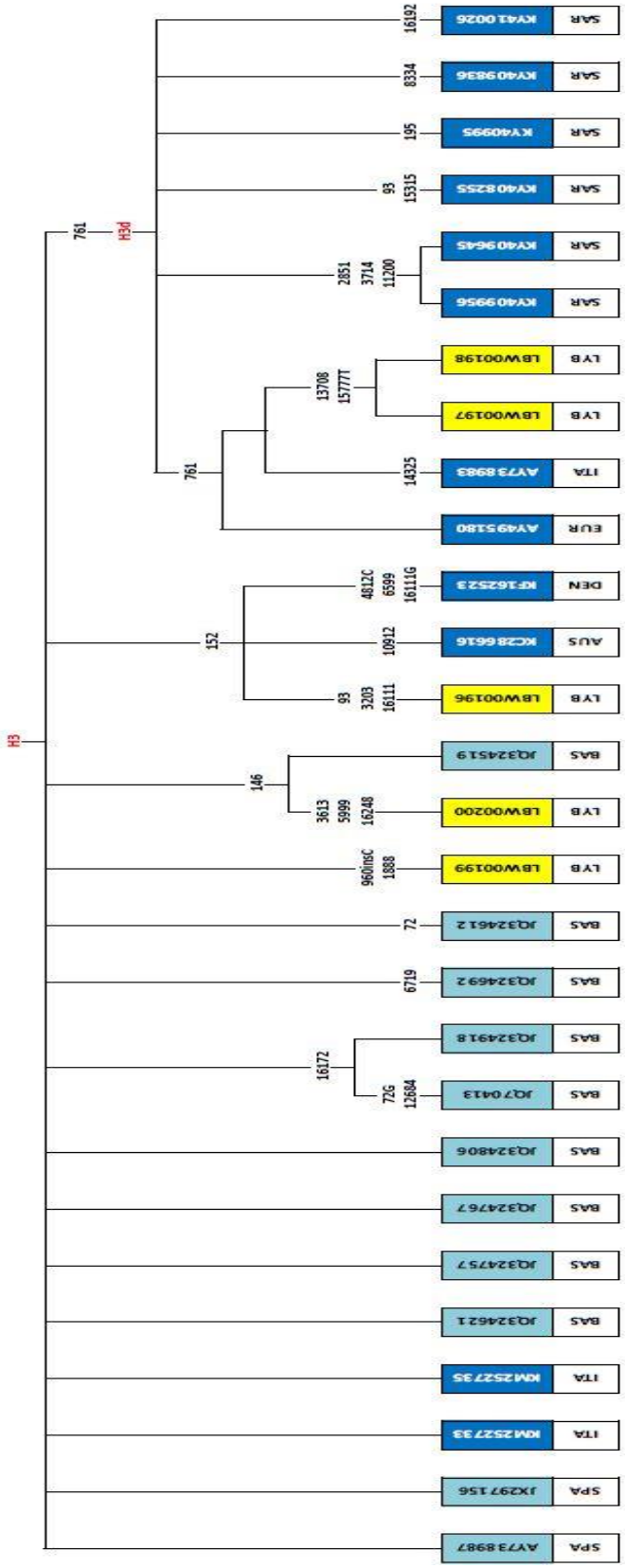

Figure 58. The tree of mtDNA complete sequences of H3 lineage

(Libyan samples in yellow, European in blue and light blue for Iberian (DEN, Denmark; LYB, Libya; Bas, Basque; SPA, Spain; AUS, Austria; ITA, Italy; SAR, Sardinia; UNK, Unknown)

4.1.2.3.5. Haplogroup H14a

Haplogroup H14a characterised HVS-I motif C16256T–T16352C, it originated in the Near East and is mostly found in Western Europe. The phylogeographic distribution of this clade indicates an ancient relationship between Western Europe and Near East through migrations along the Mediterranean coast (Figure 59). H14 has been dated to 22.8 kya, pre-LGM, before the Neolithic expansions period and 16.8 kya for H14a (Barral-Arca et al., 2016). Current phylogeographic analysis of H14a members shows the presence of this clade mainly in the Mediterranean European coast (Barral-Arca et al., 2016).

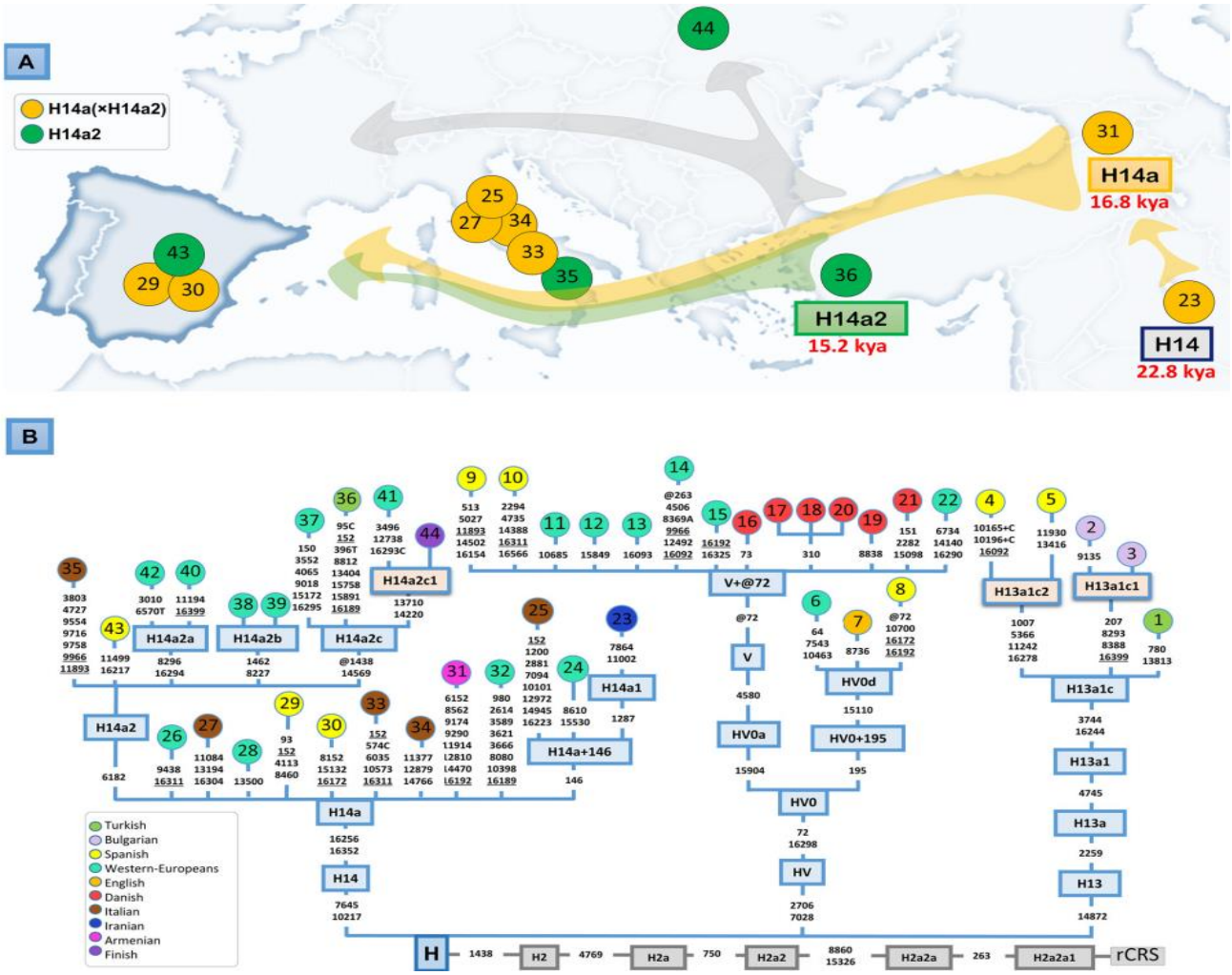

Figure 59. Dispersal along the Mediterranean and the tree of haplogroup H14a

Taken from (Barral-Arca et al., 2016)

The H14a clade was also represented in Libyan dataset by a sample from western Libyan Arab. The majority of samples in this clade are Italian from Sardinia, Figure 60.

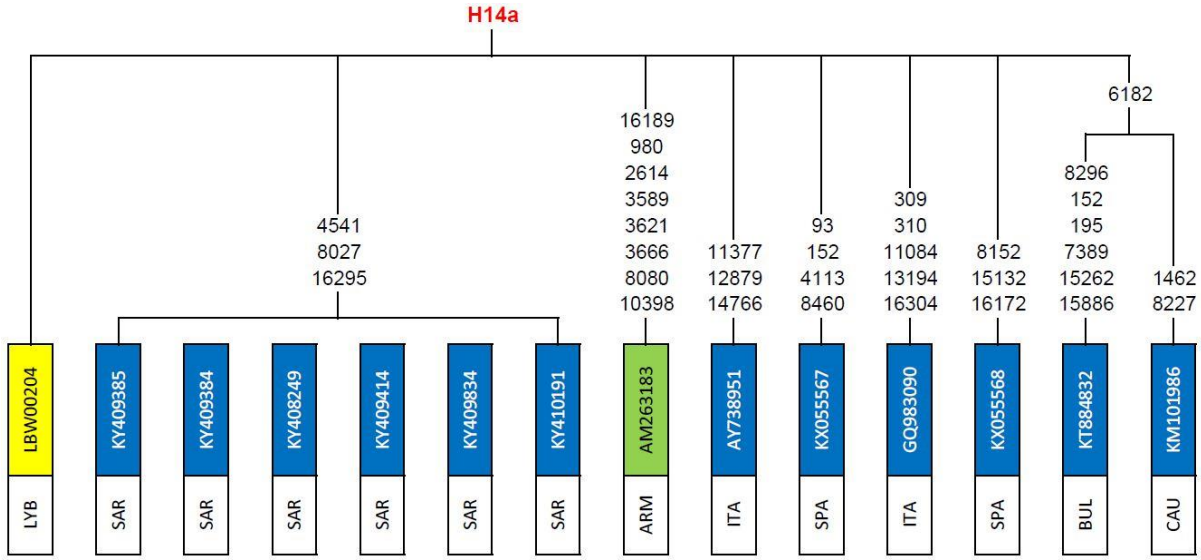

Figure 60. The tree of mtDNA complete sequences of H14a lineage

(Libyan samples in yellow, European in blue and light green for Near East (LYB, Libya; SPA, Spain; ARM, Armenia; ITA, Italy; SAR, Sardinia; BUL, Bulgaria; CAU, Caucuses)

Some other observed sub-clades of haplogroup H found less frequently in Libyan population such as H6, H2, H7 and H5 are more frequent and typical of the Near East, the Caucasus, and Central Asia. Appendix, Table 18.

Founder analysis was also performed in this study assuming that Europe and the Near East are the sources of these lineages and Libyan populations are the sink (Figure 61). The results of founder analysis showed the presence of a clear peak at 7.3 kya that may indicate dispersals into Libya at least since Holocene or possibly associated with gene flow of haplogroup V to the region (Torroni et al., 1998) in later Neolithic time (Rando et al., 1998).

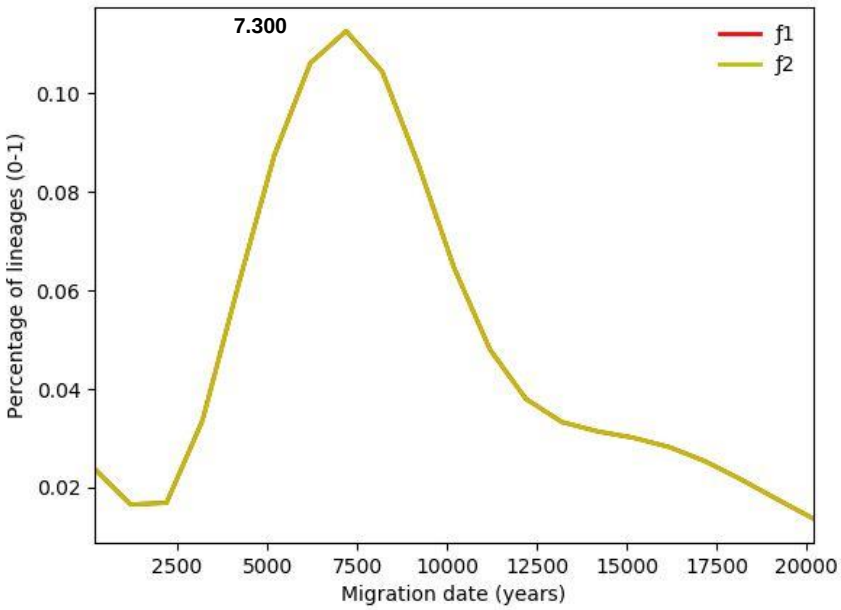

Figure 61. Founder distribution of H lineages across migration times

4.1.2.3.6. Discussion

These results supported the finding of the previous study in Libya where H haplogroup was 17.2% (Fadhlaoui-Zid et al., 2011b). Subhaplogroups H1and H3, are two frequent subhaplogroups of H, accounted 49% of all haplogroup H, dated to ~11 kya in Libya in this study and by (Ottoni et al., 2010) to 14.7 kya (Cherni et al., 2005, Ennafaa et al., 2009, Fadhlaoui-Zid et al., 2011a). These haplogroups show high frequency in Iberia and North Africa especially in Moroccan Berber (Achilli et al. 2004). However, the percentage of H1 is higher in the western part of North Africa, which is similar to the Iberian Peninsula and less frequency in the eastern region. The presence of these clades in Libya and North Africa points the importance of the Franco Cantabrian refuge area as a main source of the populations that repopulated northern and central Europe when climatic conditions began to improve ~15 kya post-glacial wave (Roostalu et by al., 2007) and probably indicates that these European hunter-gatherers also moved toward the south, crossing the Strait of

Gibraltar, contributed their H1, H3, mtDNAs to modern North Africans (Achilli et al., 2005). This is also supported by frequency patterns and the coalescence age of haplogroup V (Torroni et al. 2001a). The age of H1 among Libyan Tuareg is associated with the period of decline of the Garamantes (ancestors of Tuareg) between 2.7 and 1.8 kya (Ottoni et al., 2010). Also, comparison of mtDNA H haplogroups in the Libya population with Egypt and the Arabian Peninsula revealed gradual distribution between Western and Eastern North African population. This differentiation in frequency and distribution of H subclades between Libyan and Eastern populations shows affiliation of Libyan mitochondrial gene pool to the other North African populations. Fadhlaoui suggested that the differences found between Western and Eastern North Africa to the presence of a corridor along the Nile Valley, which might have allowed the contact between Egypt and Eastern Africa; influencing only slightly the rest of North Africa (Fadhlaoui-Zid et al., 2011b). On another hand, the Near Eastern contribution to the H lineages in North Africa is limited compared with Iberia, which leads to increasing the diversity among ancient Near Eastern population gene pool in North Africa that was initially constituted by African back lineages U6 and M1 (Harich et al., 2010). Finally, the high percentage of H1 and H3 in the north-west edge of North Africa decreases eastwards and there is a gradient of their ages between Near East 18 kya, Iberia 14 kya and North Africa 11 kya that probably points to a late Palaeolithic settlement for these haplogroups in North Africa. Overall, the younger age of H1 and H3 lineages in Libya and North Africa support that North Africa was the receiver of these lineages rather than the origin place as most of the H lineages in Libya are likely linked to the post-glacial expansion from the Iberian Peninsula rather than a recent event such as Arabic expansion.

4.3.3. Haplogroup R

The two sister clades J and T entered into Europe during the Neolithic ~10 kya. These clades diverged in the Near East (Richards et al., 2000a, Soares et al., 2009), ~43 kya for J and 29 kya for T within the timeframe of the settlement in the Levant (Figure 62) (Pala et al., 2012). It has been suggested that the J and T lineages spread from the Near East to Europe ~19 kya during the peak of the last glaciation, accompanied with the major expansions of Europe population in the Late Glacial period (Pala et al., 2012).

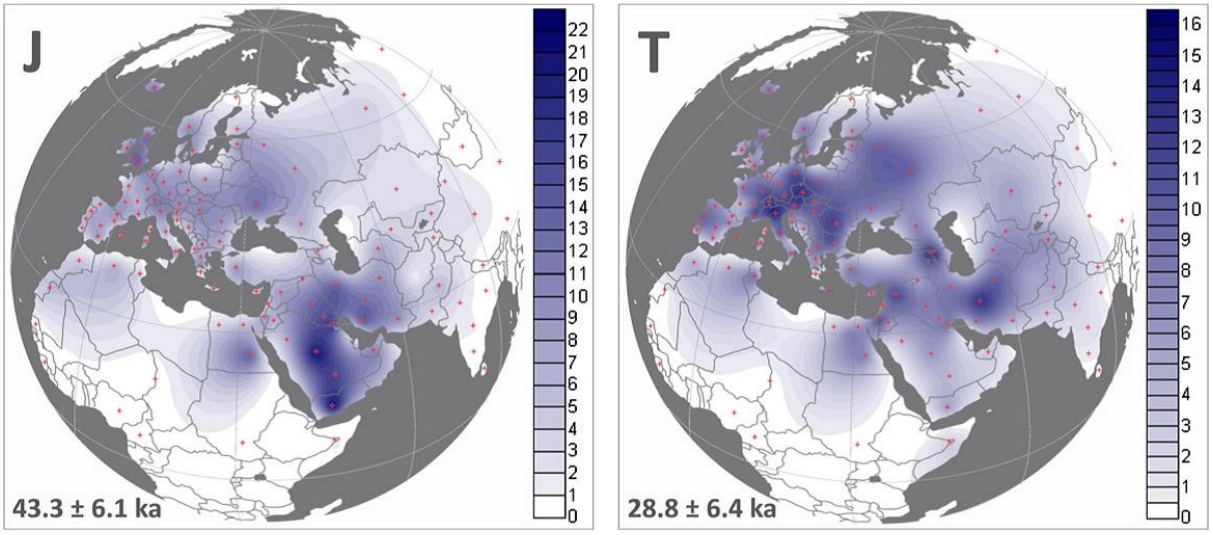

Figure 62. Spatial frequency distribution maps of haplogroups J and T

4.3.3.1. Haplogroup J

Haplogroup J makes up almost 9% of mtDNAs in Europe and ~13% in the Near East represents 10.7% of the Libyan population in this study. It has also been suggested that haplogroup J was diversified in the Near East from a single lineage originating in the North Caucasus characterised by a transition at16193 (Pala et al., 2012).

4.3.3.1.1. Haplogroup J1

Haplogroup J1 is characterised by two transitions at 462 and 3010 dated to 33 kya by Pala et al., 2012 (Figure 63). Haplogroup J1 represents the majority of the haplogroup J occupy (7.4%) of the Libyan population dated in this study to 32.7 kya.

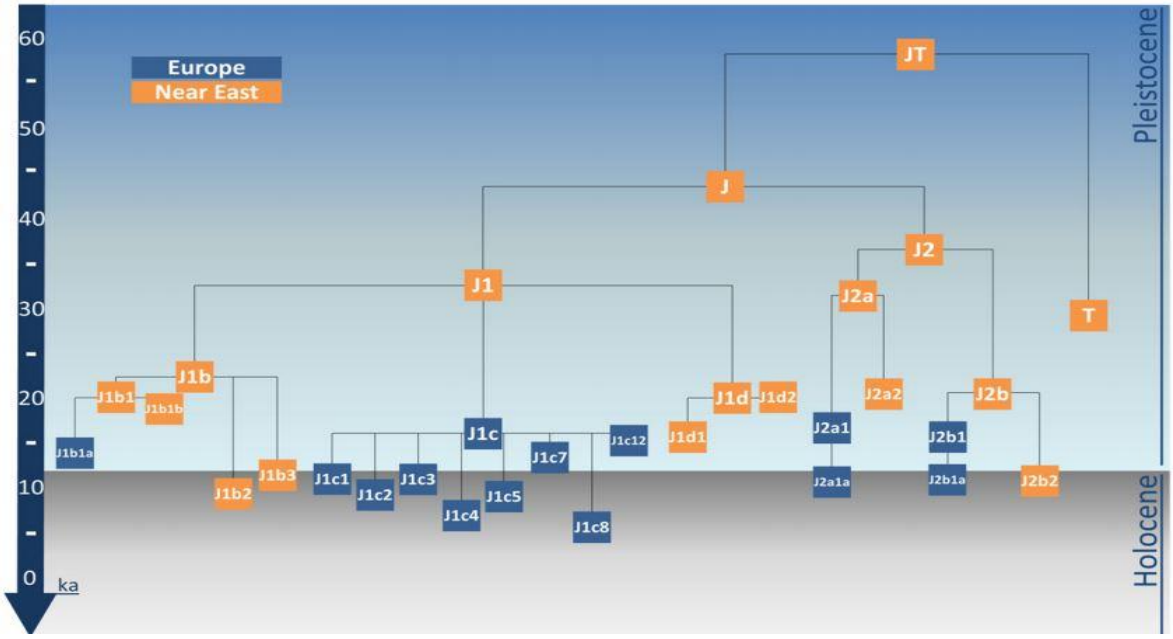

Figure 63. The phylogenetic tree of complete mtDNA sequences for Haplogroup J
From Pala et al., 2012

4.3.3.1.1.1. **Haplogroup J1b**

J1b is characterised by a mutation at np 8269, 16145, 16222 and 16261. It is predominantly a Near Eastern subclade originated ~23 kya found especially in Iran and Arabia (Pala et al., 2012). J1b is the most abundant subclade in the Arabian Peninsula aged by Abu-amero et al to a Palaeolithic time of ~29 kya (Abu-Amero et al., 2008). However, the data obtained in this study was 26.6 kya. Libyan sequences in this clade are Arab representing 2 % of Libyans carrying transition 1733 sharing a cluster with Tunisian, Moroccan and Italian in addition to other Eastern samples. Libyan samples in this clade were dated to 8.4 kya (Figure 64).

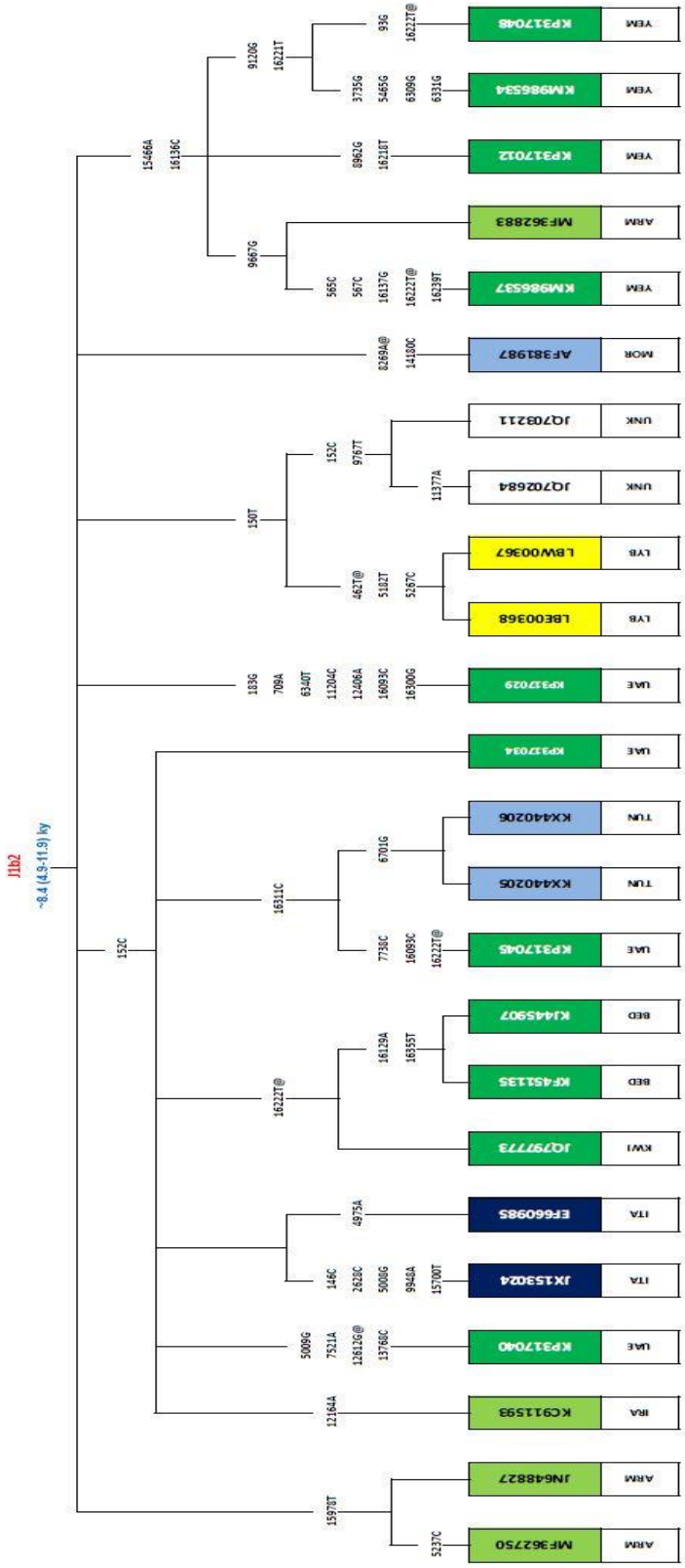

Figure 64. Tree of complete mtDNA sequences of J1b2 lineage.

Libyan samples in yellow, North African in sky blue, Arabia in green, Near East in light green, and European in blue. (LYB, Libya; ARM, Armenia; ITA, Italy; YEM, Yemen; UAE, Emirates; TUN, Tunisia, BED, Bedouin; MOR, Morocco; IRA, Iran)

The J1b1 sub-clade is defined by transitions at 5460 and 13879 dated to 22.9 kya (Pala et al., 2012) and 19.5 kya in this study. In addition, a transition at 271 defines a J1b1b branch dated to 16.6 kya in this study, was also dated to 17.6 kya (Pala et al., 2012) and between 8.5 kya and 15.2 kya (Behar et al., 2012b). The Libyan samples in this branch are either Eastern Arab or individuals from the Ghadames population shared a branch with Palestinian, Emirates, Iranian and Indians branch dated to 14 kya is Figure 65.

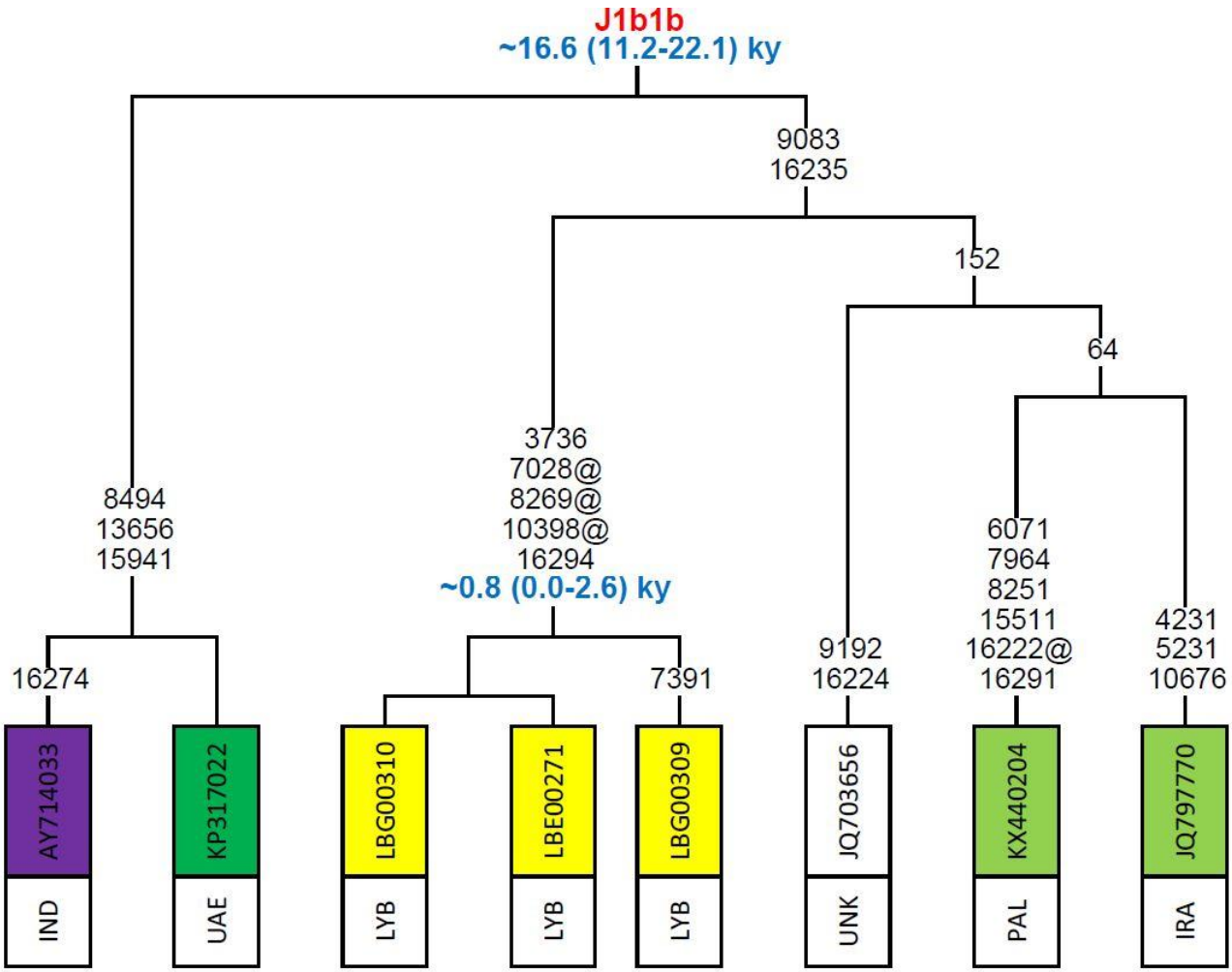

Figure 65. Tree of mtDNA complete sequences of J1b1b lineage

(Libyan samples in yellow, Arabia in green, Near East in light green, and Asia in purple)

(LYB, Libya; UAE, Emirates; PAL, Palestine; IND, India; IRA, Iran)

4.3.3.1.1.2. haplogroup J1c

The J1c clade is defined by transitions at 185, 228, and 14798, was found initially in Central Europe, the Balkans and Ukraine dated to 16 kya (Pala et al., 2012). J1c is also found in the Near East; however, it has been suggested that J1c reached the Near East as a result of a back migration lineage (Pala et al., 2012). It is also found in Neolithic and Mesolithic remains from individuals from Spain, Germany, France and Sweden and has been dated to 5 kya (Pala et al., 2012). J1c was dated in this study to 16.7 kya. The J1c1 branch of J1c is defined by transitions 482 and 3394 represents 32% of Zuwara Berber dated in this study to 11.1 kya, suggesting it originated in the Upper Palaeolithic age. All other the samples in this branch are European, lie in the root of the tree (Figure 66). Comparing with the phylogeny of global human mtDNA variations on PhyloTree.org, several new polymorphic positions were found in this study at np (5082, 7627 and 15580) suggesting a new branch within J1c1 subclade.

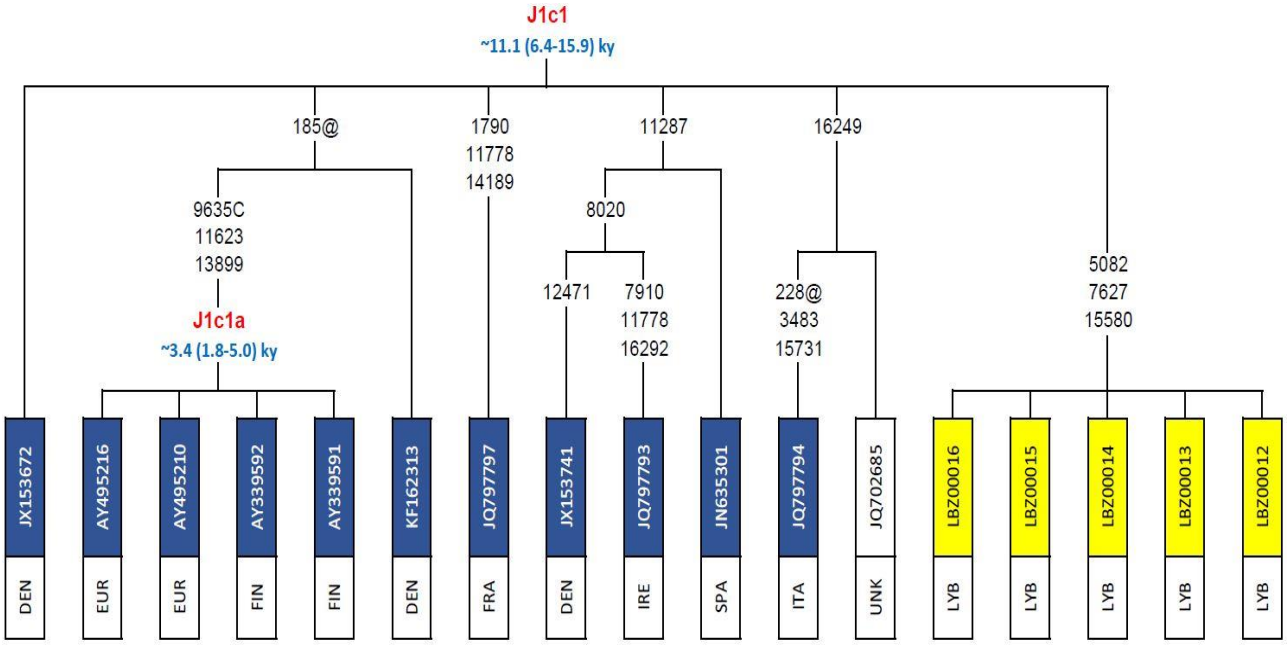

Figure 66. Tree of complete mtDNA sequences of J1c1 lineage

(Libyan samples in yellow, Europe in blue)

LYP, Libya; DEN, Denmark; FIN, Finland; IRE, Ireland; ITA, Italy; SPA, Spain; FRA, France)

4.3.3.1.1.3. Haplogroup J1d

J1d is defined by transitions at 152, 7789 and 7963 and is found predominantly in Near Eastern populations and extends from Arabia into eastern Africa, Iran, the North Caucasus, to Central Asia (Richards et al., 2000a, Maca-Meyer et al., 2001). J1d originated ~20 kya (Pala et al., 2012), and was dated in this study to 25 kya. Clade J1d1 found in several Libyan individuals is defined by a transition at 16300, dated to 20 kya in this study. All J1d1 Libyan samples in the tree belong to Eastern Arabs and Ghadames population groups represented at 9.3% and 10.7% respectively. These samples are located in branch shared with an Italian sample defined by transition at and 7789 dated to the 2 kya, (Figure 67).

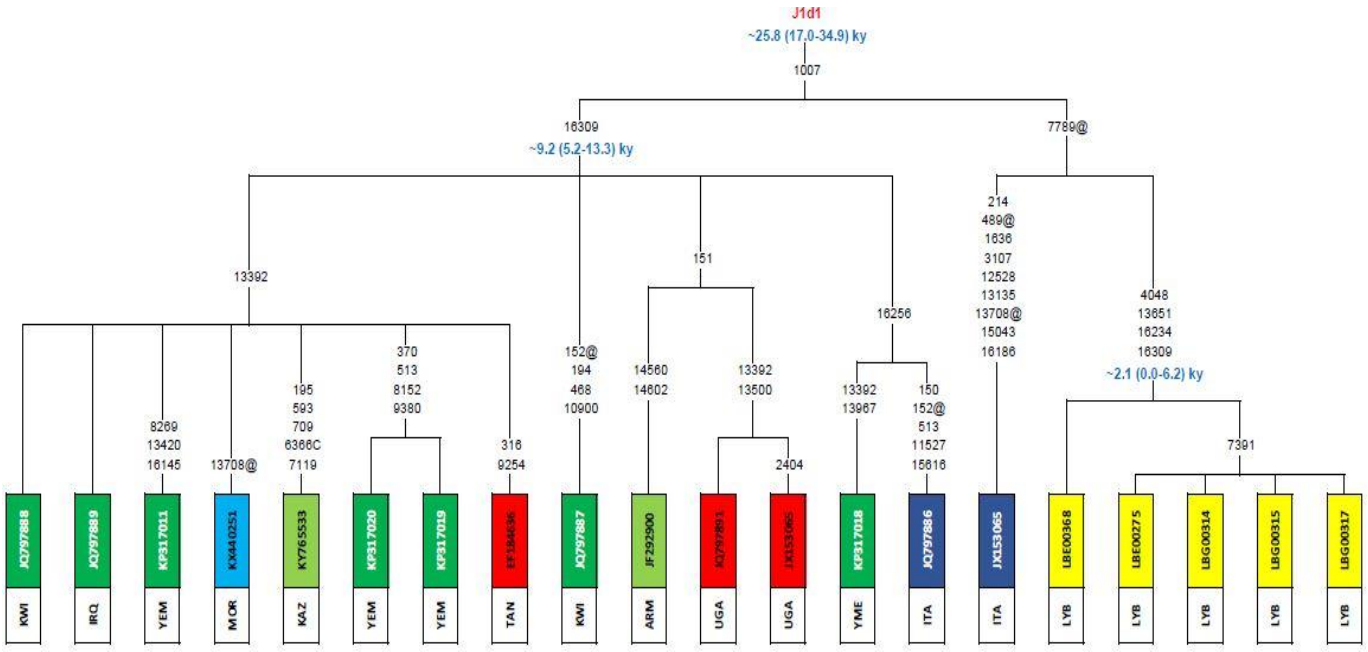

Figure 67. Tree of complete mtDNA sequences of J1d1 lineage

(Libyan samples in yellow, Europe in blue, North African in sky blue, Arabia in green, Near East in light green, African in red) (LYB, Libya; ITA, Italy; KWI, Kuwait; YEM, Yemen; UAE, Emirates; ARM, Armenia; IRQ, Iraq; MOR, Morocco; KAZ, Kazakhstan; UGA, Uganda; TAN, Tanzania)

4.3.3.1.2. Haplogroup J2

The haplogroup J2 is less abundant than haplogroup J1; however, it is represented at 3.3% the Libyan population and dating to ~37 kya. The two main clades of this haplogroup are J2a and J2b dating to ~32 and ~20 kya respectively. Although several J2 clades are found in Europe such as J2a1 and J2b1 date to 15.5 kya, J2 clades are predominantly Near Eastern (Pala et al., 2012).

Haplogroup J2a2 is a branch has dated to 16 kya (Behar et al., 2012b) and 18.7 kya (Pala et al., 2012). These subclades also spill into north-eastern Africa and north-western Africa, while J2a2b is found throughout North Africa and J2a2d is also found in Algeria and the Canary Islands (Pala et al., 2012).

Libyan samples in this study were belonging to Eastern Libya found in the J2a2c branch defined by transition at 16231 with Arabian subjects. This branch J2a2 has been dated to 20 kya (Pala et al., 2012) Other Libyan samples in this study were located in the base of the J2a2 clade while the Fadhlaou's samples were classified as J2a1 and J2b (Fadhlaoui-Zid et al., 2011b) (Figure 68).

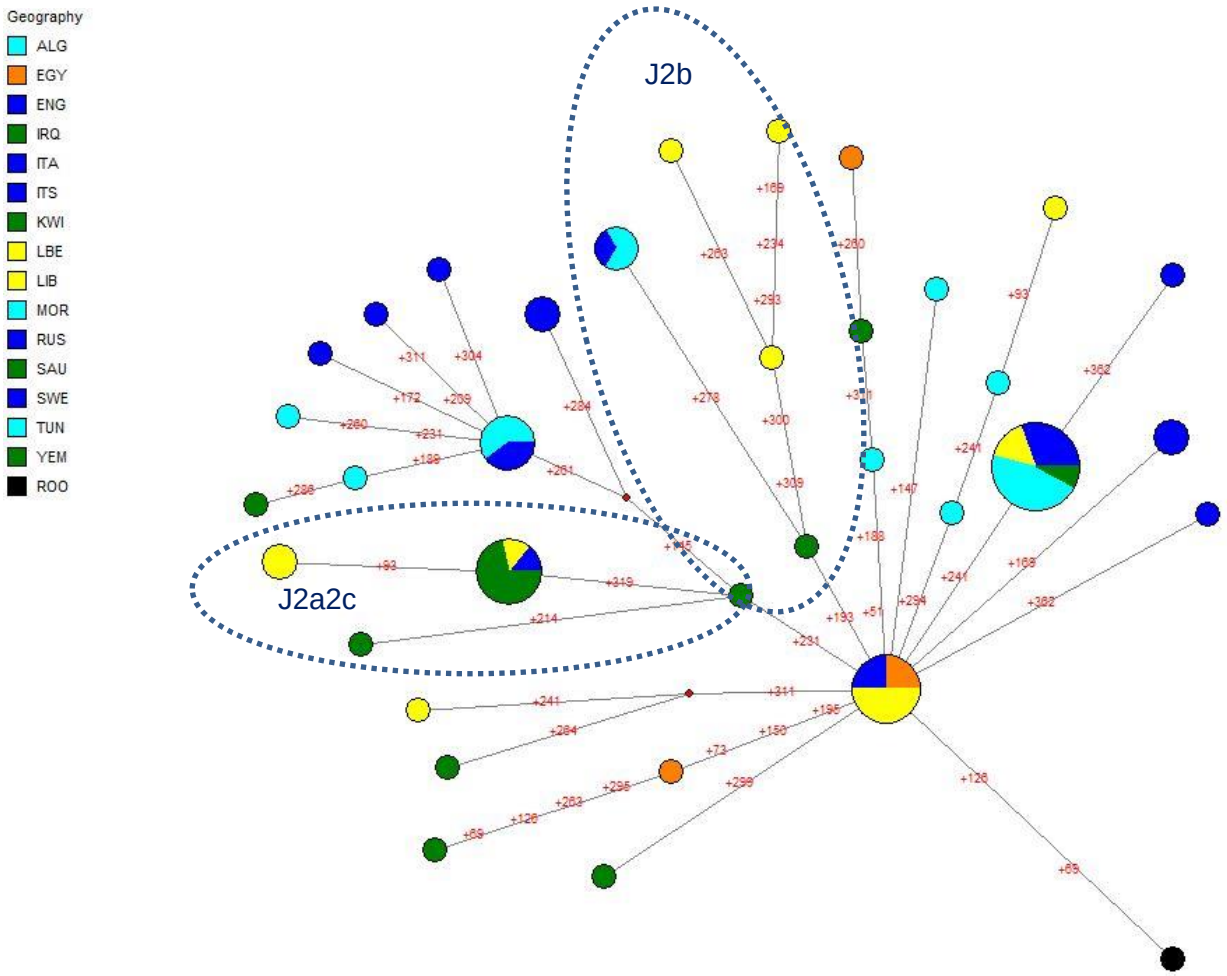

Figure 68. The HVS-I reduced-median-network for subclade J2 for Libyan groups

(Libyan samples in yellow, Europe in blue, North African in sky blue, Arabia in green, Egypt in orange and the root in black) (LIB Libyan Fadhlaoui et al., 2011, LBE Libyan this study; ITA, Italy; IRQ, Iraq; YEM, Yemen; SAU, Saudi; TUN, Tunisia; MOR, Morocco; ITA, ITS, Italy; RUS, Russia ENG, England KWI, Kuwait; ALG, Algeria; SWE, Sweden).

Founder analysis was performed for haplogroup J assuming that Europe, Arabia and the Near East are the sources of this lineage and Libyan populations are the sink (Figure 69). The results of founder analysis showed the presence of dispersal at 8 kya that may indicate dispersals into Libya during Capsian culture in North Africa in the Mesolithic time.

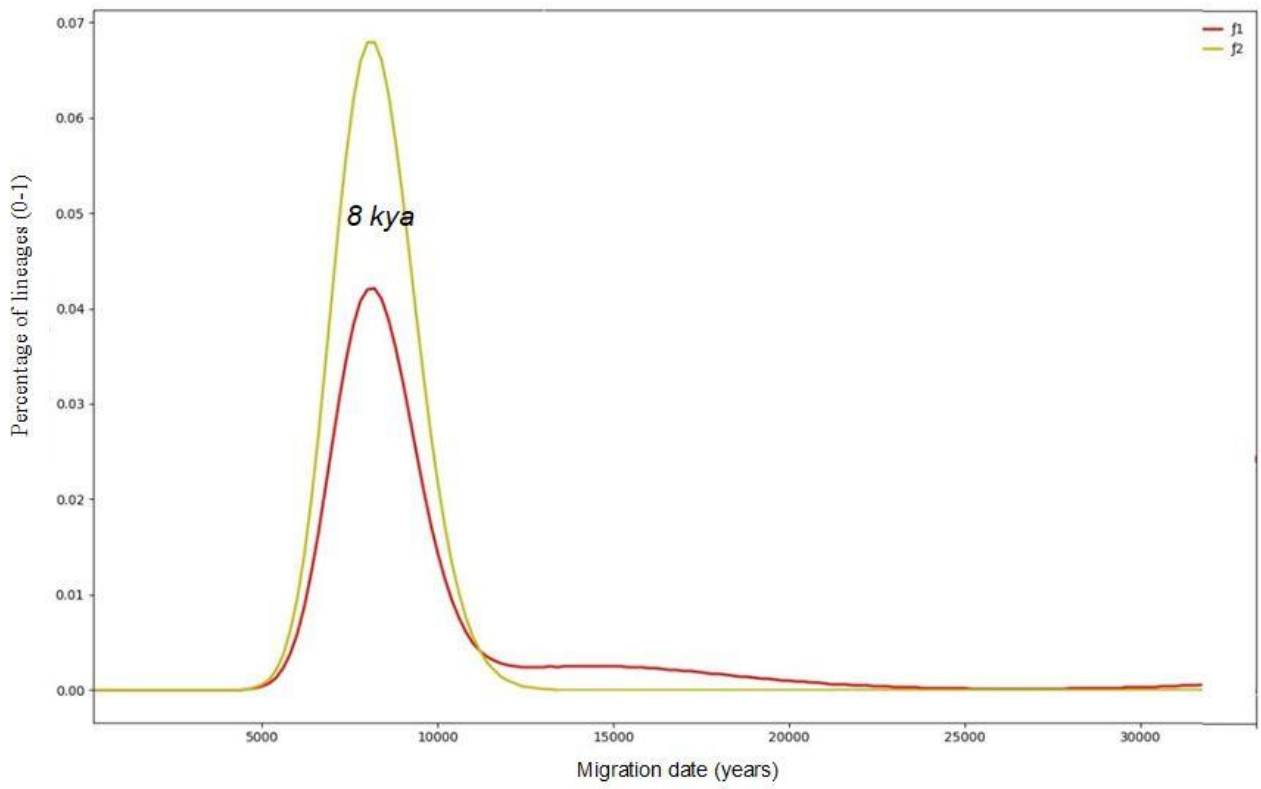

Figure 69. Founder distribution of J lineages across migration times

J lineages harbour several clades originated in different regions around the world as can be seen in the J1b2 tree. Subhaplogroup, J1b2 is found in Moroccan, Tunisian, Libyan, Italian and Spain. The phylogeographic distribution of this clade indicates an ancient relation between Mediterranean countries and Near East, suggesting that the lineages arose in the Near East (Pala et al., 2012) and migrated along both Mediterranean coasts during the Mesolithic time. Like J1b2 clade, the J1b1b Lineages originated in Near East in LGM, before the Neolithic expansions period 17 kya (Pala et al., 2012) and arrived in Libya with Capsian culture 8 kya. The European clade J1c dated to 16 kya represented 80% of J1 lineages in Europe. However, it is found in the Near East and North Africa at low frequencies. It has been suggested that these lineages reach the Near East as a back migration. Basque county contains the highest percentage of J1c in Europe at 11% (Cardoso et al., 2011). J1c1 was also found at high frequency among Coastal Berber Zuwara at 32%. The high percentage of this haplogroup among Berber population may explain by drift given the level of isolation of this population. The low frequency of this clade in Near East and presence of J1c in

other North African population may indicate that the source of J1c in North Africa is the Iberian Peninsula rather than Near East, suggesting that this lineage arrived along with other Franco Cantabrian lineages H1, H3 and V through Strait of Gibraltar.

J1d found in the wide range from central Asia, Near, North Caucasus, East to Arabia and eastern Africa. As can be seen in the tree in Figure 70, it is present in Armenia and Italy, suggesting this lineage arrived through the Mediterranean coast.

The predominantly Near Eastern J2 represented in North Africa by J2a2b and J2a2d was found in Libya and other North African countries and is present in the Canary Islands. J2ac2 is another clade in this haplogroup was also found in Libya. This rare clade is only found in Yemen and Sardinia. J2b which evolved in Southeast Europe during the Late Glacial was also found among the Libyan population (Fadhlaoui-Zid et al., 2011b).

The presence of these different J lineages among the Libyan population and other North African population indicating the presence of several migration waves through history. Some of the lineages are of Neolithic origin like J1b2, and other lineages such as J1b1 may have reached Libya by diffusion of Islam. The J1c clade seems to have reached North Africa from Iberia or Levant in the last glacial maximum period.

4.3.3.2.Haplogroup T

Haplogroup T also originated in Near East and had been dated to 30 kya. T lineage accounts for almost 8% of mtDNA in the Near East and 10% in Europe (Pala et al., 2012). Haplogroup T represented 4.6% of the Libyan population and was dated in this study to 27 kya. This subhaplogroup contains two subclades, T1, and T2 that diverged in the Near East at 21 kya, and each of these clades includes several lineages. T1 is widely distributed from the Near East, Northwest Africa, Europe, and Caucuses. Among T1 there is major clade named T1a dating to 17

kya representing the vast majority of T1. This is also widespread represents 8% of the Tunisian gene pool.

Samples belonging to the T1a6 sub-clade a major clade T1a were found in this study and was restricted to Costal Berbers from Zuwara representing ~ 4% among them. The widespread clade T1a dating to ~15.6 kya is represented at 9% in Romania, 8% in Tunisia, 6% in northeastern Iran, and ~5% in South Caucasus. Zuwara Berber subjects shared a branch with Tunisian, Moroccan and Emirates sequences (Figure 70). The samples within T1a are characterised by transitions at 3867 and 10376 that define T1a6 clade dated to 1.8 kya by (Behar et al., 2012b) and found to 2 kya in this study (Figure 70).

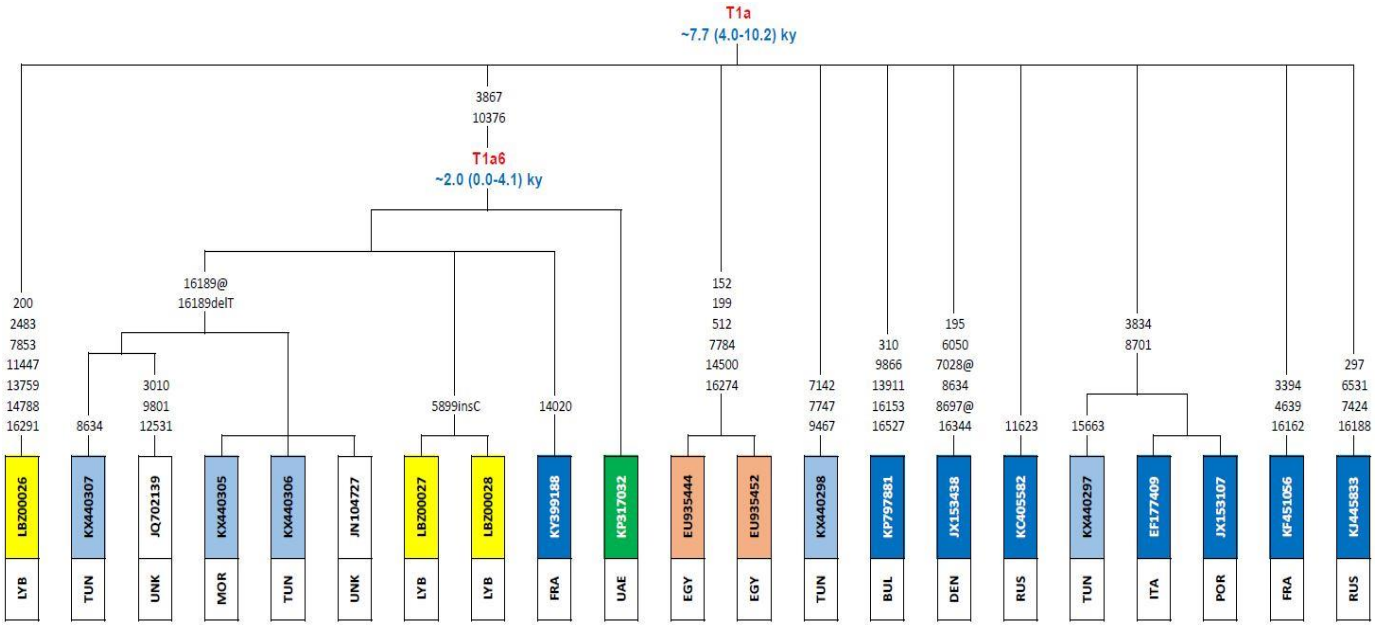

Figure 70. Tree of complete mtDNA sequences of T1a lineage

Libyan Zuwara Beber samples in yellow, North African in sky blue, Egypt in orange, Arabia in green, and Europe in blue (LYB, Libya; TUN, Tunis; MOR, Morocco; FRA, France; UAE, Emirates; EGY, Egypt; BUL, Bulgaria; DEN, Denmark; RUS, Russia; ITA, Italy; POR, Portugal)

Haplogroup T2 is predominantly found in central and Western Europe and in the Mediterranean reaching 80% of total T in Western Europe, dating to ~21 kya. It also represents 10% of samples found in the Levant and Iran (Pala et al., 2012). It is dated in this study to 21.7 kya. In this study, T2 is represented by T2c1a subclade which accounts for 3.6 % of Libyans which is dated in this study

to 15 kya and is found in Eastern Arab and Ghadames samples. The Libyan samples clustered in the branch are defined by a transition at 16296 dated to 1.7 kya. This branch also contains samples from North Africa, Arabia and Near Eastern populations (Figure 71).

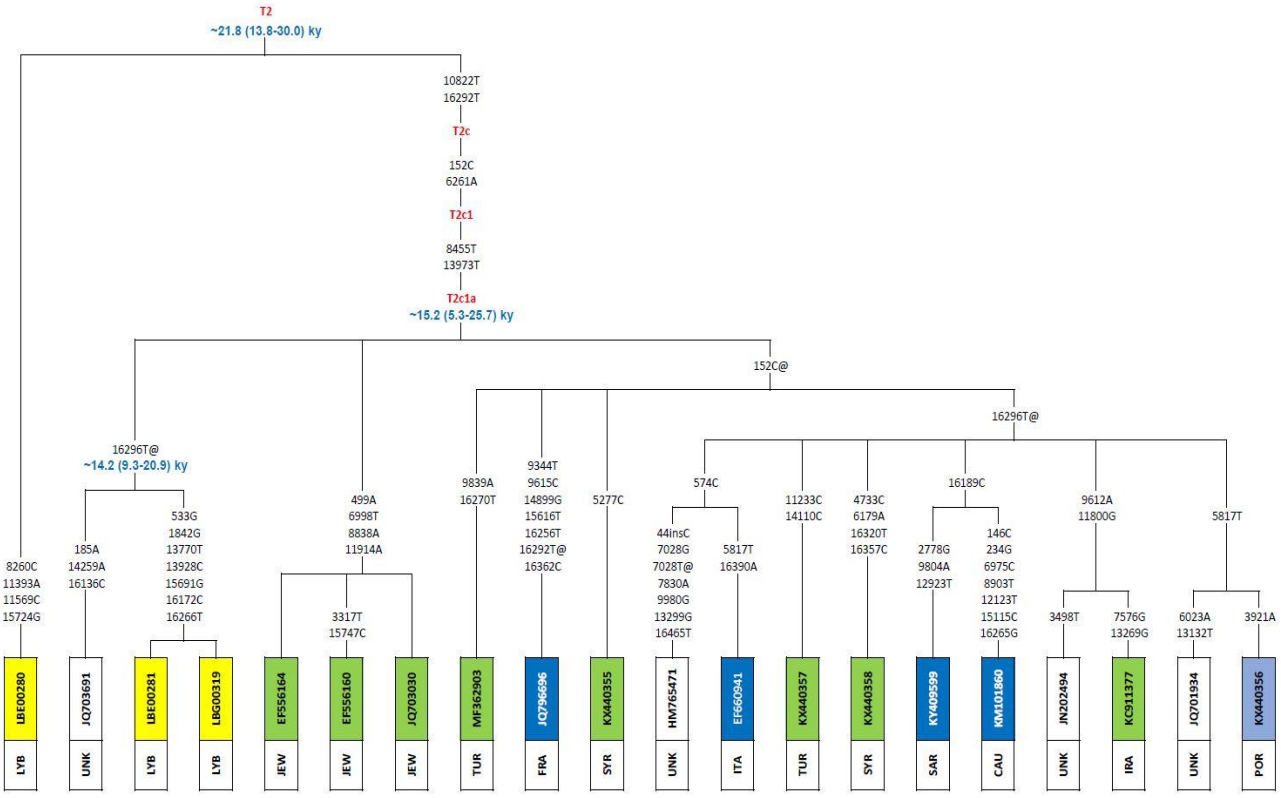

Figure 71. Tree of complete mtDNA sequences of T2 lineage

Libyan samples in yellow, Europe in blue, Near East in light green, Iberian in Light blue) (LYB, Libya; JEW, Jewish; TUR, Turkey; FRA, France; SYR, Syria; IRA, Iran; CAU, Caucuses; ITA, Italy; POR, Portugal)

Founder analysis performed for haplogroup T assuming that Europe, Arabia and the Near East are the sources of this lineage and Libyan populations are the sink Figure 72. The results of founder analysis showed the presence of dispersal at 10.2 kya indicating a population migration during the late glacial period from the Near East or at least since the Capsian culture in North Africa in the Mesolithic time led by T2.

There was also, a weak peak seen at 22 kya in the upper Palaeolithic that may refer to T1 expansion toward North Africa (Figure 72).

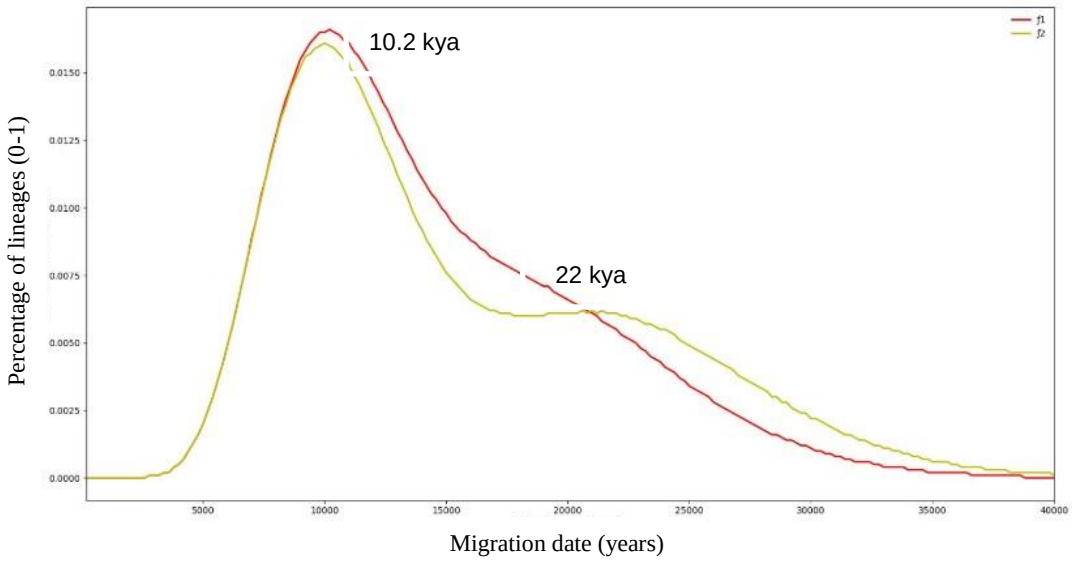

Figure 72. Founder distribution of T lineages across migration times

Since haplogroup T was found in Iberomaurusian bone remains in Morocco (Kéfi et al., 2005), the T lineage seems to have reached North Africa since the Palaeolithic period. Iberomaurusian culture in the Maghreb extends to Egypt (Barton et al., 2013). However, since there is a debate about the origin of the Iberomaurusian culture, it is difficult to know the source of Haplogroup T in the region.

4.3.4. Haplogroup U

The analysis of whole mitochondrial DNAs belonging to haplogroup U shows that haplogroup U branched out shortly after the "out of Africa" migration and rapidly branched into several regionally distinct subhaplogroups (Achilli et al., 2005). Haplogroup U is a Europeans specific clade defined by a transition at 12308. It is ancient haplogroup, with an estimated age of ~50 kya years (Torroni et al., 1996). Cluster U includes several sub-clusters (U1–U8), each having a specific geographic distribution. Thus, only haplogroups U1–U5 have been found in Western European populations. The U7 West Eurasian-specific haplogroup is typical in the populations of Jordan, Kuwait, Iran, and Saudi Arabia (Abu-Amero et al., 2007b).

4.3.4.1. Haplogroup U5

U5 is one of the most ancient European mitochondrial DNA (mtDNA) haplogroups since the Upper Paleolithic; it is common in Western Europe and the Mediterranean and also occurs in the Middle East. U5 which is represented in 7% of Europe populations migrated into Europe in the Upper middle Paleolithic around 36 kya (Torroni et al., 1996, Richards et al., 2000a). Two U5 subclades were identified, U5a, and U5b, each dating to ~27 kya indicating that both clades have at least originated before the Last Glacial Maximum (Malyarchuk et al., 2010). Analysis of mtDNA HVSI, in Europeans, showed the presence of a cluster of U5 aged 12 kya or even less. It has been suggested that the expansions of such U5-subclades are powered by climatic changes during the Holocene, resulting in the migration of the Southern population in Europe to occupy a wide area of the northern part of Europe (Malyarchuk et al., 2010) (Figure 73).

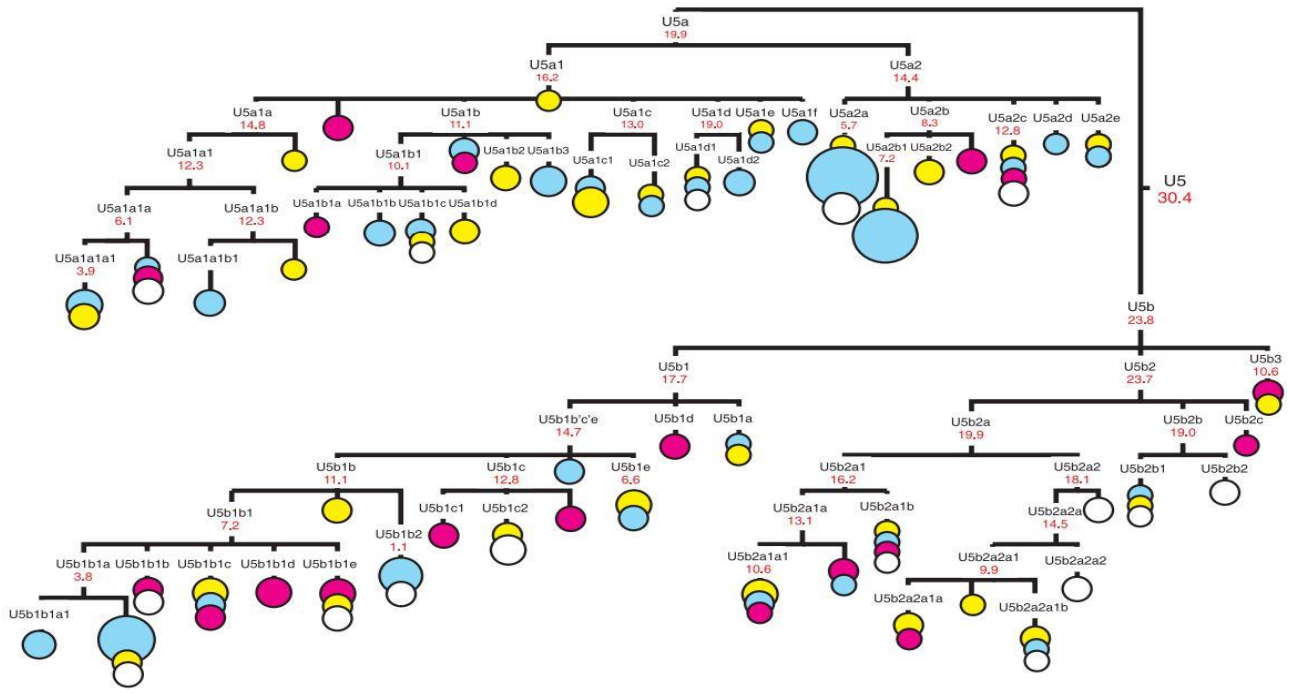

Figure 73. Complete mtDNA phylogenetic tree of haplogroup U5.

(Eastern European in blue, central European in yellow, Mediterranean and western European in fuchsia, white are unknown) Taken from (Malyarchuk et al., 2010)

U5a arose during the LGM between 16 and 20 kya. U5a is also further divided into two phylogenetic clusters, U5a1 and U5a2. U5a1 and U5a2 are thought to have expanded shortly after the LGM. In addition, U5a2 is frequent in Eastern and central Europe and has been detected in the Mediterranean populations including Tunisians and Italians dating to 18 kya.

U5a2 was detected in this study among two Berber populations from the Nafusa mountain and the Zuwara coastal town. Both of these samples were located in clade shared with North African and European samples, the Libyan samples from Fadhlaoui et al., 2011 were branches from branch shared between European and Arabian samples (Figure 74).

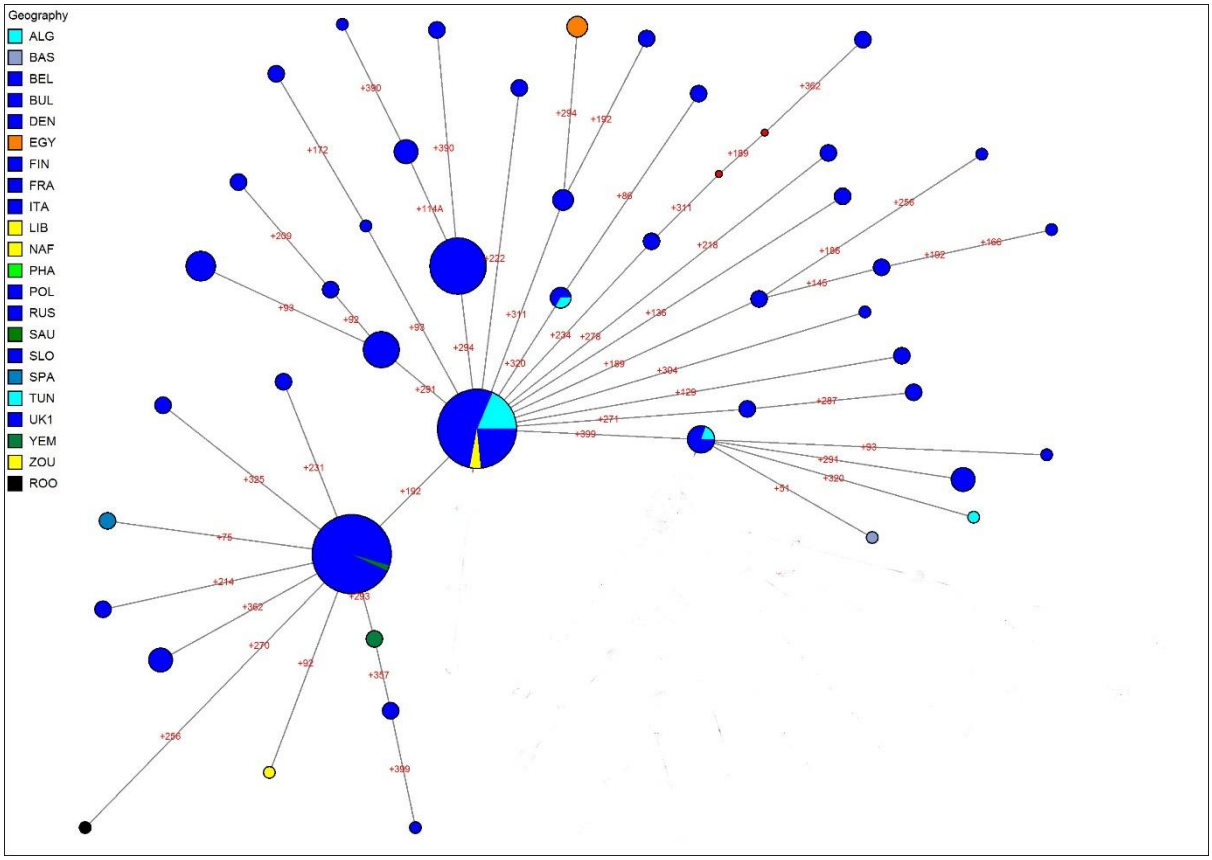

Figure 74. The HVS-I Reduced-median-network for subclade U5a for Libyan groups

(Libyan samples in yellow, Europe in blue, North African in sky blue, Arabia in green, Egypt in orange, and root in black) (LIB Libyan from Fadhlaoui et al., 2011, ZOU, Zuwara, NAF, Nafusa mountain; ITA, Italy; BAS, Basque; BEL, Belarussian; EGY, Egypt; FIN, Finland; FRA, France; PHA, Palestine; POL, Poland; SLO, Slovakia; SPA, Spain; TUN, Tunisia; BUL, Bulgaria; DEN, Denmark; ALG, Algeria; YEM, Yemen; SAU, Saudi; RUS, Russia; UK1, England; ALG, Algeria).

4.3.4.1.1. Subhaplogroup U5b

Subhaplogroup U5b initially diverged in southern and central Europe 27 kya before LGM time and then spread to Eastern Europe. It is subdivided into U5b1, U5b2 and U5b3. While U5b2 is dated to 18 kya, the remaining clades are dated by lower ages. U5b1 is represented in Libya by U5b1e which is mainly present in central Europe by Hungarians, Slovaks, Czechs and southern Russians (Malyarchuk et al., 2010). U5b1e was found amongst the Ghadames population. U5b2 was also found among Berber populations in Libya. Zuwara and Nafusa Berber samples were represented by U5b2b. Libyan samples are located in the tree within a branch defined by transitions at 16296 and 13161 shared with Sardinian individuals. (Achilli et al., 2005) (Figure 75).

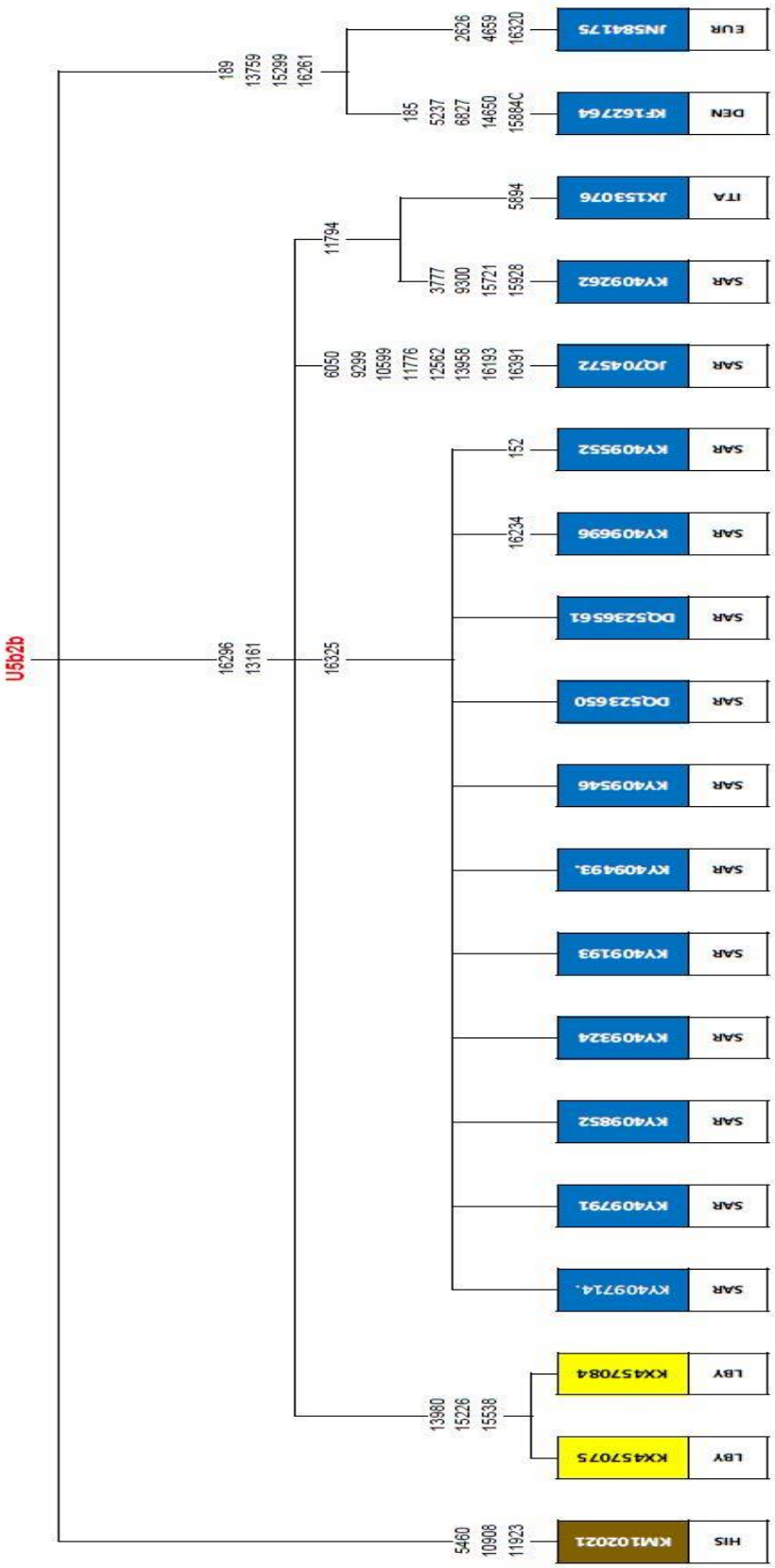

Figure 75. Tree of complete mtDNA sequences of U5b2b lineage

Libyan samples in yellow, Europe in blue, USA, Brown) (LYB, Libya; ITA, Italy; SAR, Sardinia; HIS, Hispanic

4.3.4.1.2. Subhaplogroup U1a

U1a clade is common in the Southwest, and South Asia arose in Southwest Asia between 29 and 44 kya. U1a split early into several subclades U1a1, U1a2, U1a3, and U1a4) dated to 13-15 kya. It is also found in the Caucasus and Europe (Derenko et al., 2013). The Western Libyan sample in this clade is located in Persian-specific branch (Iran) within U1a3 defined by a transition at 7403 named by Derenko as U1a3a and dated to 8–10 kya (Derenko et al., 2013). The U1a3a branch is also detected among Sardinian population indicating a long-standing relationship between Mediterranean populations (Figure 76).

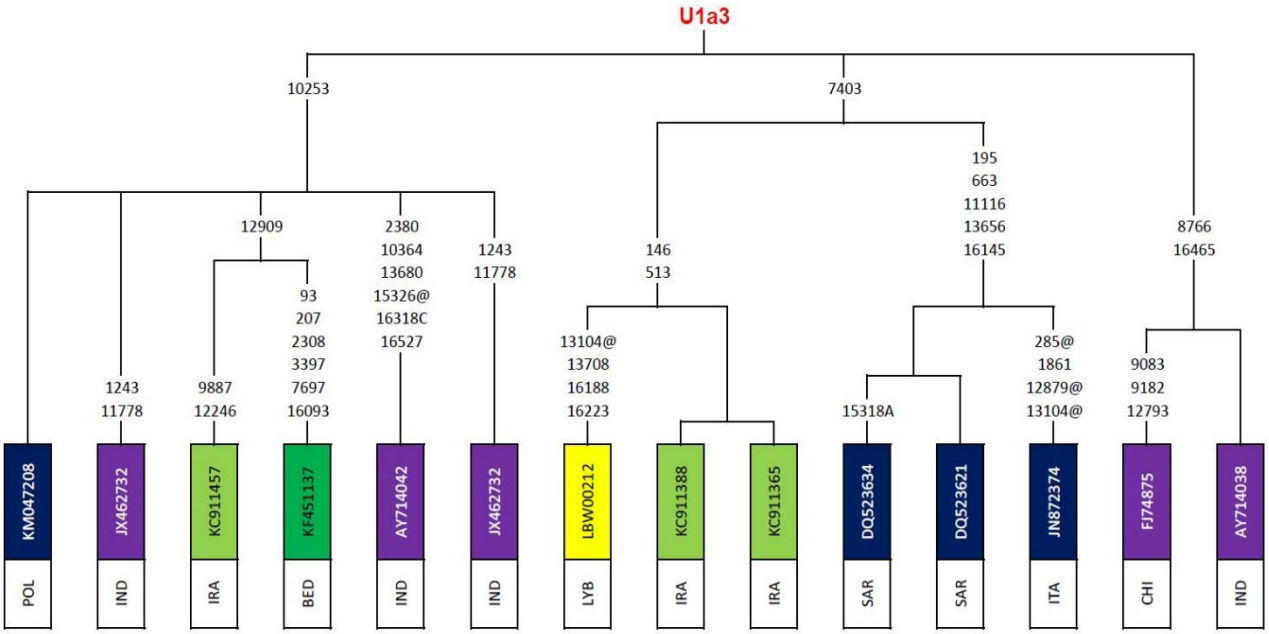

Figure 76. Tree of complete mtDNA sequences of U1a3 lineages

Libyan samples in yellow, Europe in blue, Asia in purple, Near East in light green (LYB, Libya; ITA, Italy; SAR, Sardinia; POL, Poland; IND, India; CHI, China; BED, Bedouin; IRA, Iran)

4.3.4.1.3. Subhaplogroup U7

U7 is the youngest clade within haplogroup U that originated in Near East 18.6 kya, then shortly dispersed to South Asia 11.5 kya before the Holocene (Sahakyan et al., 2017). U7 lineages also have a recent dispersal toward Mediterranean Europe during the early Holocene. U7b exhibits a

higher level of diversity in the Mediterranean and southeastern of Europe (Sahakyan et al., 2017) (Figure 77).

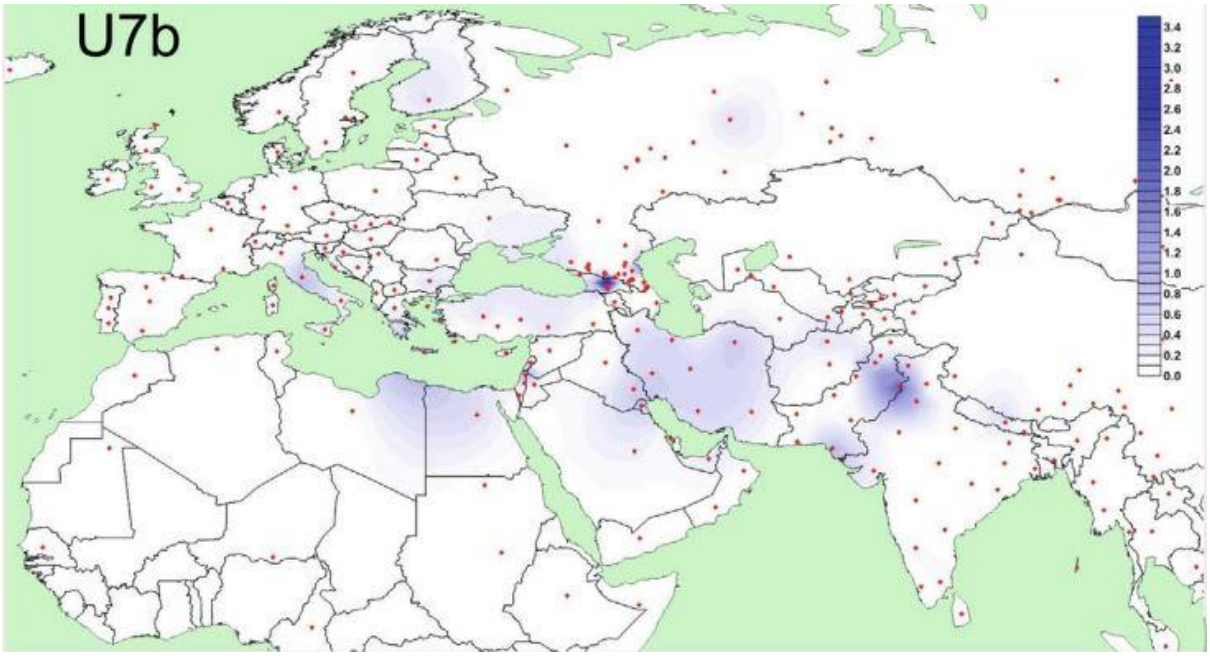

Figure 77. Frequency distribution maps of haplogroups U7b

Taken from (Sahakyan et al., 2017)

Two western Libyan samples within U7 are represented by clade named U7b1b, dated to 14.4 kya (Sahakyan et al., 2017) that share a branch with Kuwaiti sample defined by transitions at 16184 and 16381 dated to 13 kya in this study (Figure 78).

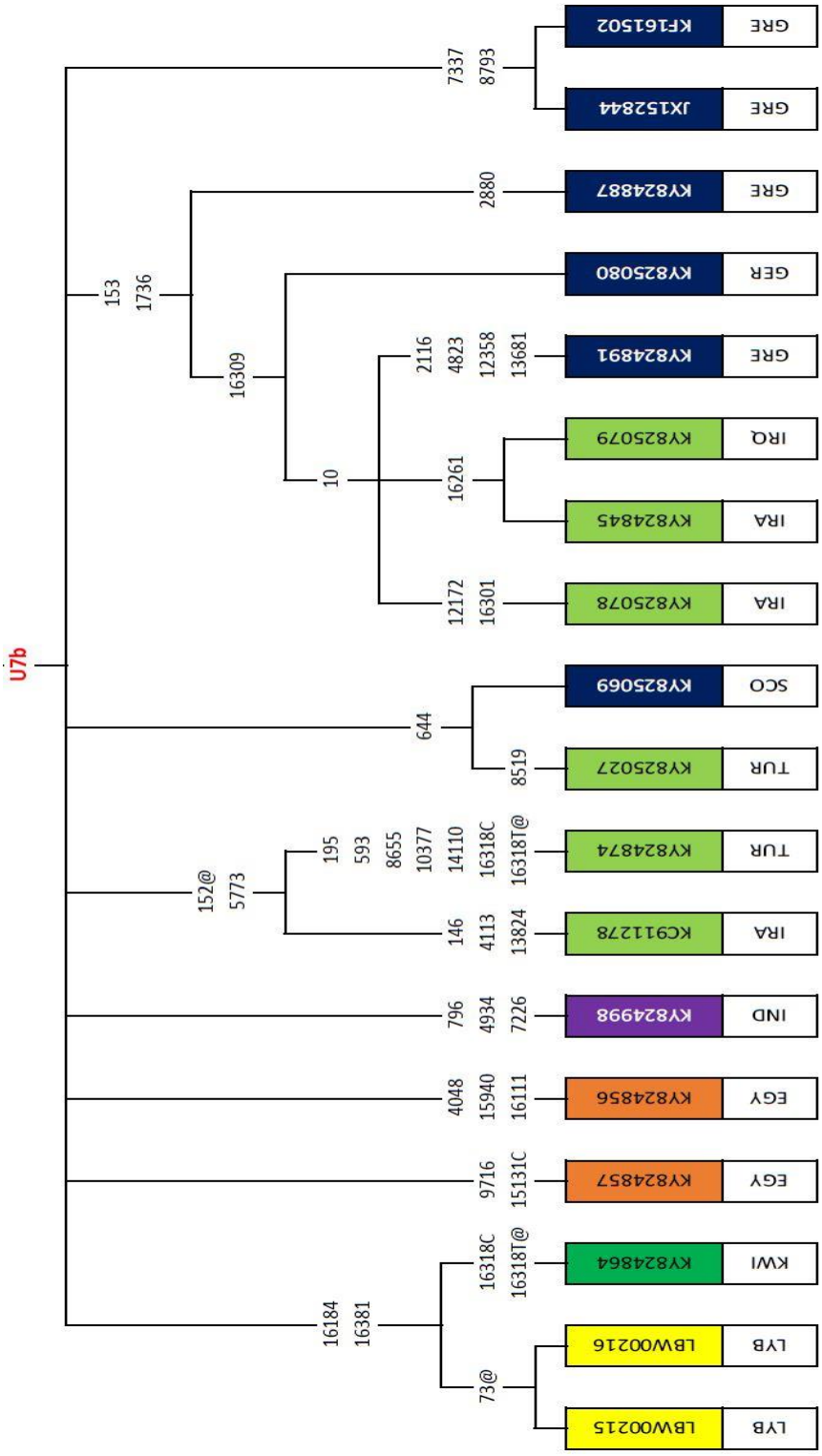

Figure 78. Tree of complete mtDNA sequences of U7 lineage

Western Arab Libyan samples in yellow, Europe in blue, Asia in purple, Near East in light green, Egypt in Orange (LYB, Libya; EGY, Egypt; Kuwait; TUR, Turkey; SCO, Scotland; IRQ, Iraq; IND, India; GRE, Greece; GER, Germany; IRA, Iran).

Other observed subclades of haplogroup U found in less frequency in Libyan population such as U2, U3, U4 and U8 are listed in Appendix table 18.

Like haplogroup J, U lineages harbour several clades originated in different regions around the world. As can be seen in the U trees (Figure 75, 76 and 78). The European U5 lineage is restricted to Berber groups in Libya. U5 is also found among other North African population such as Tunisian and Morrocan Berbers and is rare in Egypt (Coudray et al., 2008), Levant and Arabia. Furthermore, Achilli et al. stated that frequency patterns and ages of some U5b clades found in North African Berber and Iberia are similar to haplogroups H1, H3 and V (Achilli et al., 2005). This finding may further confirm the role of the Franco-Cantabrian refuge as not just a major source of the hunter-gatherer in Northern Europe, but also a source of ancestral Berbers who migrated toward the south by crossing the Strait of Gibraltar when climate began to improve in late-glacial expansions at 15 kya enriched the North African mtDNA gene pool by adding haplogroups V, H1, H3 (Achilli et al., 2005).

Haplogroup U5 was found in Berber group in Libya while Eastern European U3, and another Arabian Peninsula U4 and U7 in this study were restricted to Arabic group in Libya. Furthermore, confinement of the presence of Haplogroup U5 on the Berber population and the Eastern lineages on Arab population confirm the proximity of the Berber to North African population and maybe the Europeans, while the restriction of the eastern lineages on Arabic group may indicate the Arabian peninsula origin of this group. Again the presence of Western European, Near Eastern and Mediterranean lineages of U haplogroups in the Libyan population is yet another result that confirms the presence of several founders from different origins that contributed together to build the current Libyan gene pool.

4.3.1.1. Haplogroup K

Haplogroup K is a Late Upper Palaeolithic haplogroup. In Europe it is found at 8–10% frequency in Europeans, (Allard et al., 2004) and reaches 20% in Cyprus. It accounts for 6.7% of the Libyan population with the highest frequency in the Berber group at 12% reaching 21.4% among Mountain Berbers from Nafusa. Haplogroup K arose from haplogroup U8 by two transitions at control region at 16224, 1631 and three others in coding region 10550, 11299 and 14798. It has been dated in the Near East and Europe and to 36 kya, also dated by Soares to 31.6 kya (Soares et al., 2010). It also has been suggested that haplogroup K diversified by the last glacial time in parallel in both Europe and the Near East Figure 79 (Costa et al., 2013a).

Subhaplogroup K1a

K1a defined by a transition at 497 is dated to 18.4 kya by Behar et al., 2012b and 20 kya by Costa et al., 2013 spread separately in Europe and Near East. The coalescence date for K1a is estimated in this study at 20 kya. The majority of Libyan samples were classified as K1a clade falling into different branches of K1a. K1a1b1 clade which is mainly restricted to Europe is also found in North Africa. This clade migrated from the Near East in the early Holocene 11.5 kya Figure 80 (Costa et al., 2013a).

All Libyan Berber samples located in this clade were dated in this study to 9.5 kya. The Berber samples shared a branch with Greek and German samples defined by 5583 and 12007 named K1a1b1g. The Libyan Arabic samples in this clade were located in different branches, one sample shared a branch defined by transition at 16189 with an Ethiopian sample, and the remaining samples were located in the branch defined by a mutation at 7897 shared with a Yemeni sample (Figure 80).

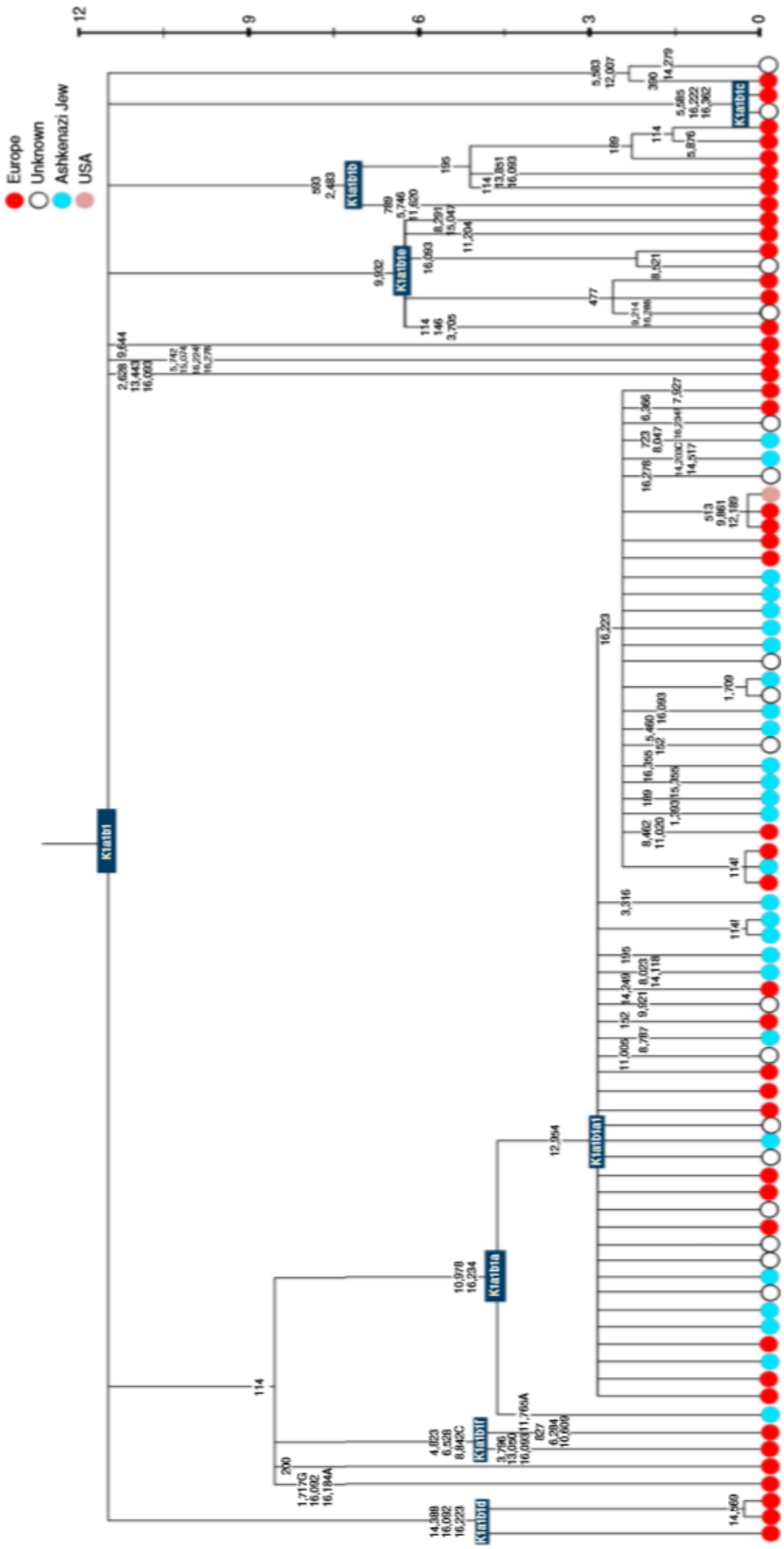

Figure 79. Tree of haplogroup K1a1b1
Ages (kya) based on ML estimations. Taken from (Costa et al., 2013a)

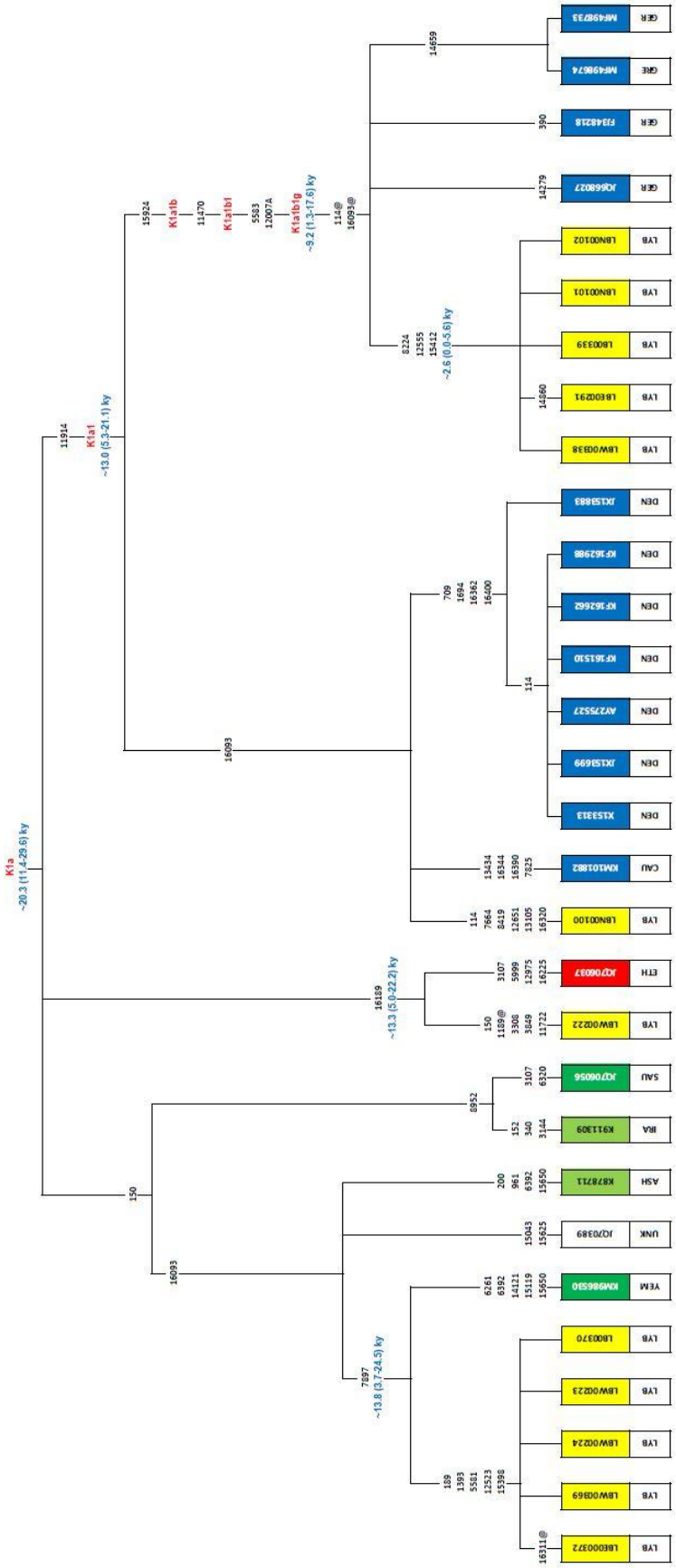

Figure 80. Tree of complete mtDNA sequences of K1a lineage

Libyan samples in yellow, Europe in blue, Near East in light green, Arabia in green, Africa in Red (LYB, Libya; ASH Jewish; YEM, Yemen; SAU, Saudi; IRA, Iran; ETH, Ethiopia; CAU, Caucuses; GRE, Greece; GER, Germany; DEN, Denmark)

Subhaplogroup K1b

K1b is identified by a transition at 5913, most likely arising in Europe, between the last glacial period and the Neolithic periods (Costa et al., 2013a). This was also found in samples in this study, represented by K1b1b. (Figure 81). Berber population from Ghadames and Nafusa and Libyan Arabs were located in the basal branch while Zuwara Berber samples located in branch shared with European samples.

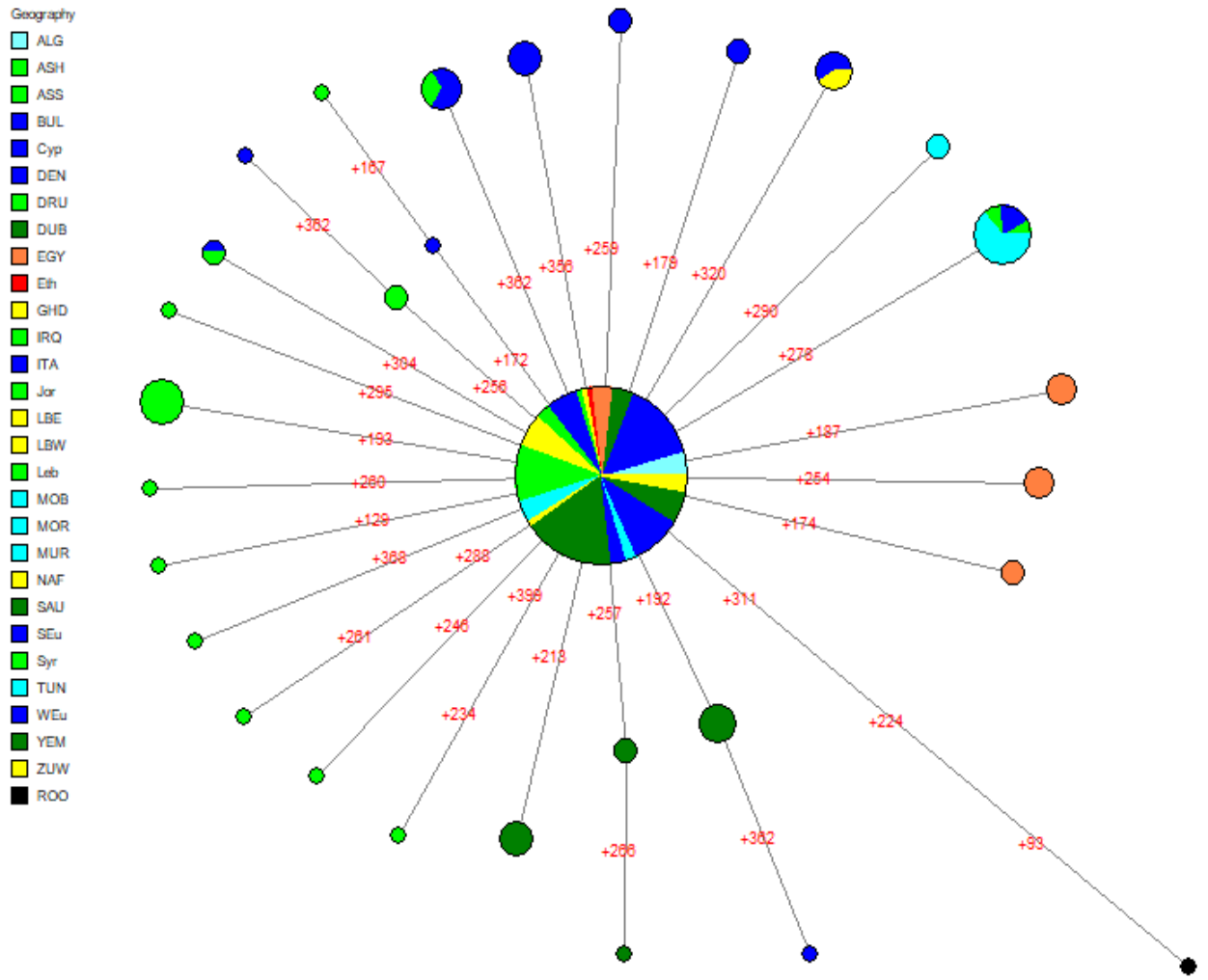

Figure 81. The HVS-I Reduced-median network for haplogroup K1b

Libyan samples in yellow, North Africa in sky blue, Egypt in orange, Europe in blue, Near East in light green, Arabia in green, Africa in Red. (LYB, GHD, LBE, LBW, ZUW, NAF Libya; MOR, MBR, Morocco; MUR, Mauritania; ASH Jewish;YEM, Yemen; SAU, Saudi; IRA, Iran; ETH, Ethiopia; SEu, South Europe; WEu, West Europe; TUN, Tunisia; ALG, Algeria; SYR, Syria; DRU, Druze; DUB, Dubai; EGY, Egypt; CH, Chad; IRQ, Iraq; ASS, Assyrian; ITA, Italy; JOR, Jordan; LEB, Lebanon; DEN, Denmark; CYP, Cyprus; BUL, Bulgaria)

K2a was also found in three subjects in this study restricted to Nafusa Berbers with a transition at 16291. K1a is defined by three mutations at 152, 709 and 4561, dated by Soares to 6.8 kya (Soares et al., 2010). Other Libyan samples belong to the basal branch of K.

Founder analysis of haplogroup K was done assuming Europe and Near East as a source of population of these lineages while Libya and North Africa are a source population. The results show a migration peak from the Near East in the early Holocene around 13.5 kya (Figure 82).

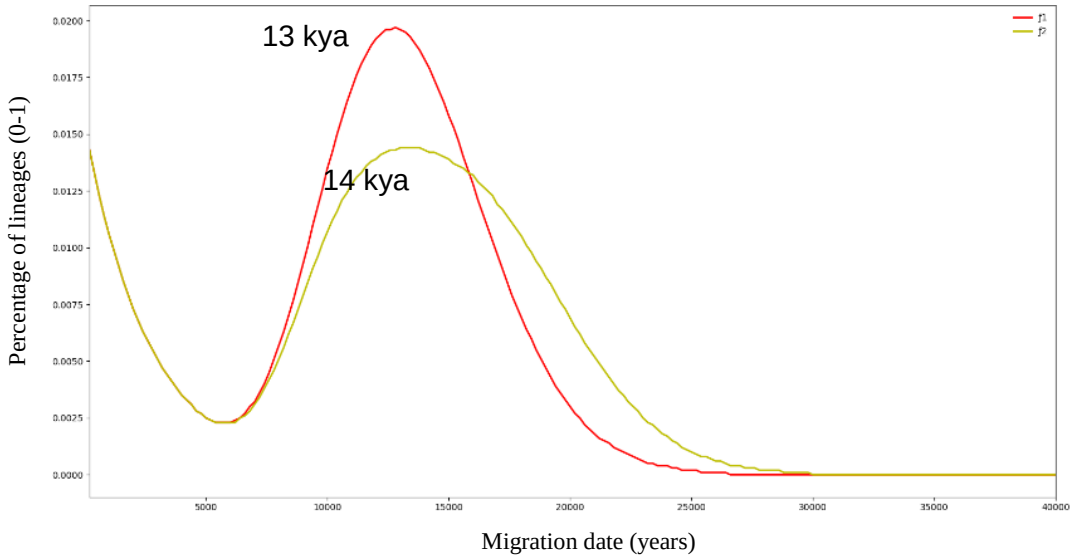

Figure 82. Founder distribution of K lineages across migration times

Haplogroup K was detected in almost all populations in Europe, Near East and North Africa. It reached its highest frequencies in Greek Cypriots and Ashkenazi (Fernández et al., 2014). The frequency of Haplogroup K in the Libyan population was 10%, reaching 21.4% among Nafusa Berbers. While the Berber sequences within haplogroup K are located with European clade K1a1b1g, Arab samples were located with other Arabian samples from Yemen and East Africa, (Ethiopia) (Figure 80).

The presence of this clade of K among Libyan Berber promotes the theory of trans-Mediterranean contacts between Europe and Northern Africa; the high percentage of this haplogroup among

Nafusa Berber population may be explained by drift gave the level of isolation of this population. The source of haplogroup K in Arabic group may be from Arabs who migrated to North Africa spreading Islam and Arabic language in recent times.

4.4. Indigenous North African Lineages in the Libyan population

4.4.1. Haplogroup U6

mtDNA haplogroup U6 termed "the main indigenous North African cluster" is a North African haplogroup that is similar to M1 of south-west Asian ancestry and is unique as it is highly distributed amongst human mtDNA haplogroups (Pennarun et al., 2012). It is found primarily in North Africa where it is at the highest frequency, the Canary Islands and near East. Its highest frequency in North Africa is in Algerian Berbers at 28%, so it has been linked to the ancestors of the Berber-speaking populations of North Africa (Maca-Meyer et al., 2003, Hernandez et al., 2014). U6 evolved from a common ancestor in the Near East, 50 kya that dispersed through the southern coast, and Cyrenaïca, during the Dabban industry, ~40-50 kya in mid-later Upper Palaeolithic, with further expansion into North Africa with the Iberomaurusian culture at ~22 kya (Macaulay et al., 2005). It is also said that this migration is associated with Afro-Asiatic linguistic Expansion (Maca-Meyer et al., 2004). U6 most likely entered Africa ~40-45 kya and evolved in North Africa (as U5 evolved within Europe (Pereira et al., 2010b). The presence of U6 lineages in the Near East probably signals a more recent gene flow from North Africa. In most of North Africa and Iberia population regions, U6 frequencies are about 10% or less and even appear absent from some Berber communities in Tunisia (Pereira et al., 2010b), and between 0.5-5% in the Iberian Peninsula (Figure 83) (Hernandez et al., 2014). U6 which is dated to 35 kya (Secher et al., 2014) accounts for 3.7% of Libyan population and reaches 10.7% and 7.8% in Ghadames and Zuwara population respectively and represents only 0.5% of Arabic group (Table 11). U6 was dated in this study to 31.3 kya.

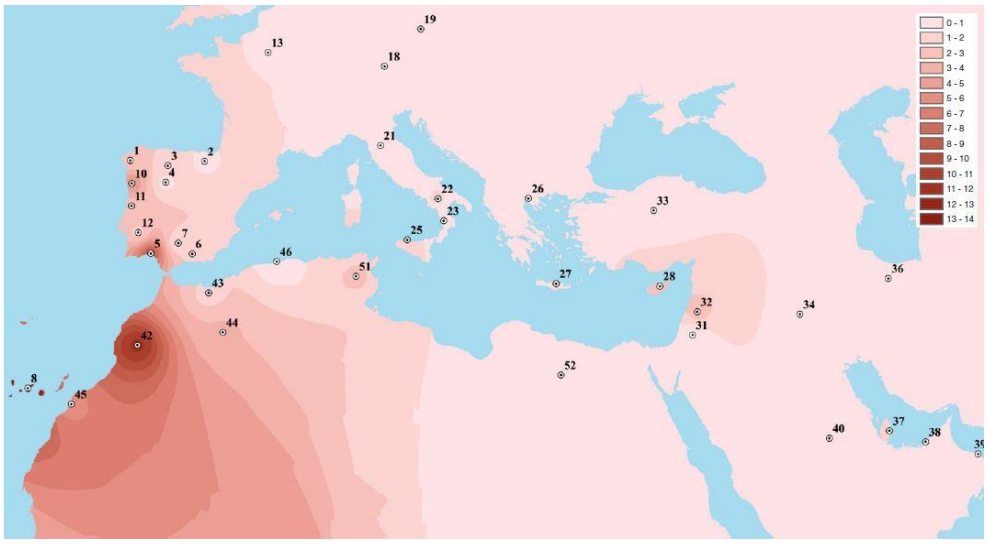

Figure 83. Frequency (%) map of lineage U6 along Mediterranean population

Taken from (Hernandez et al., 2014)

4.4.1.1. Sub-haplogroup U6a

Sub-clade U6a is the most diversified sub-clade among U6 haplogroup. That is probably due to the high diversity in this clade in Northwest Africa. The majority of U6 clades arose before the LGM, shortly after U6 33 kya (Pennarun et al., 2012). The major sub-haplogroup U6a is characterised by HVSI mutations at positions 16172, 16219 and 16278 and is highly spread throughout North Africa reaching its highest frequency in Mauritania and the Algerian Berber (Pennarun et al., 2012). Maca-Meyer et al. proposed that U6a reflect an African re-expansion from the Maghreb eastwards (Maca-Meyer et al., 2003). U6a was dated to 29.4 kya by (Pennarun et al., 2012) and 27.4 kya in this study. Within U6a, U6a8 was found in Libya represented by U6a8a dated in this study to 12.3 kya. Libyan samples in this subgroup were mainly Saharan Berber (Ghadames) and Zuwara Berber. They shared a branch with Tunisian, Moroccan Berber and Mauritanian samples. Libyan samples were located in branch defined by transition at 2775, 7813 and 9752 dated in this study to 2.5 kya (Figure 84).

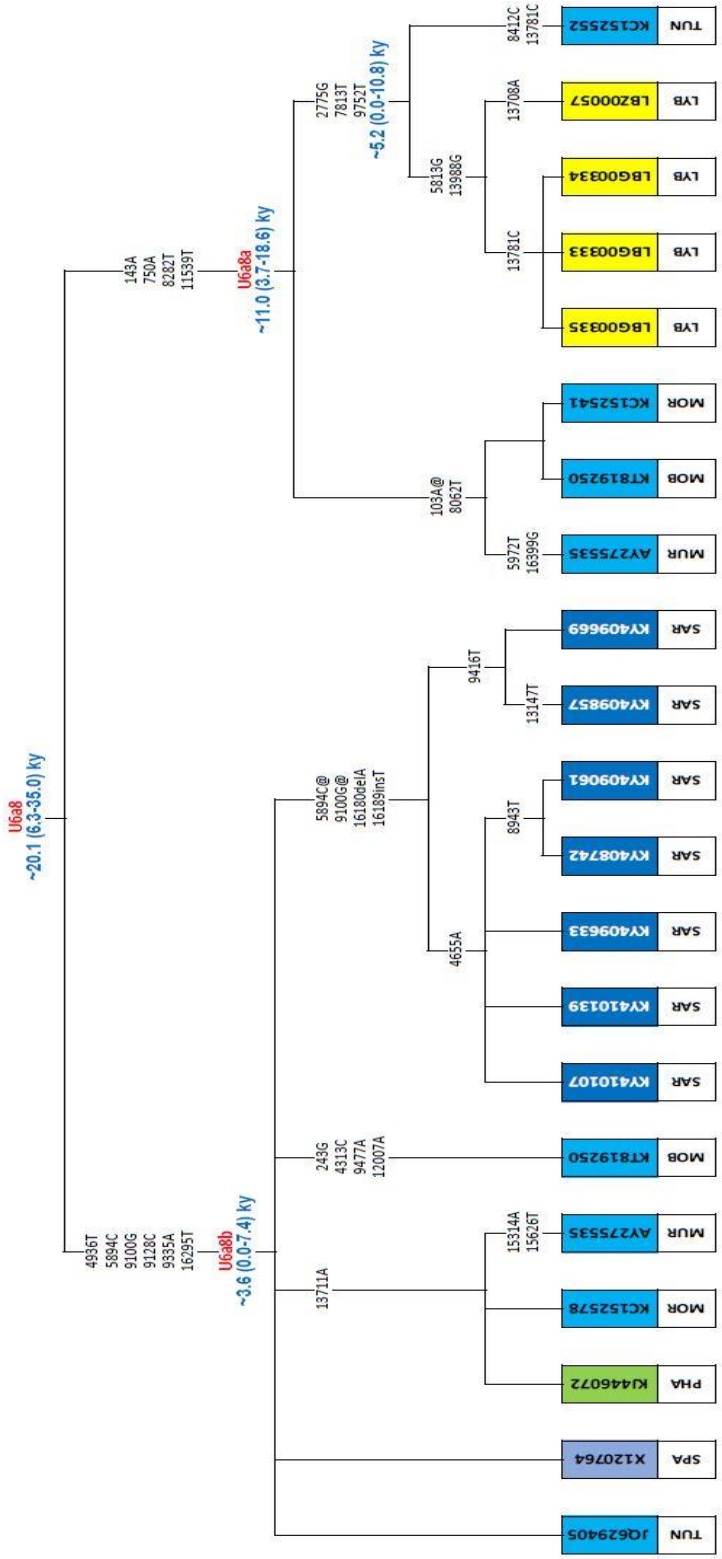

Figure 84. Tree of complete Mt DNA sequences of U6a8 lineage

Libya in yellow, North Africa in sky blue, Europe in blue, Near East in light green, Iberian in light blue (LYB, Libya; SPA, Spain; PHA, Palestine; MOR, MOB Morocco; MUR, Mauritania; SAR, Sardinia TUN, Tunisia)

Sub-groups of U6a3 were dated to 18.8 kya by Pennarun et al., 2012 and show multiple expansions via Europe by the U6a3a clade, the Maghreb by U6a3b and U6a3e clades and West Africa represented by U6a3c and U6a3f. Libyan samples in this branch were located in the two Maghrebian clades U6a3b and U6a3e dated to 2.9, and 5.2 kya in Africa (Secher et al., 2014) and 4.2 kya and 5.3 kya in this study respectively (Figure 85).

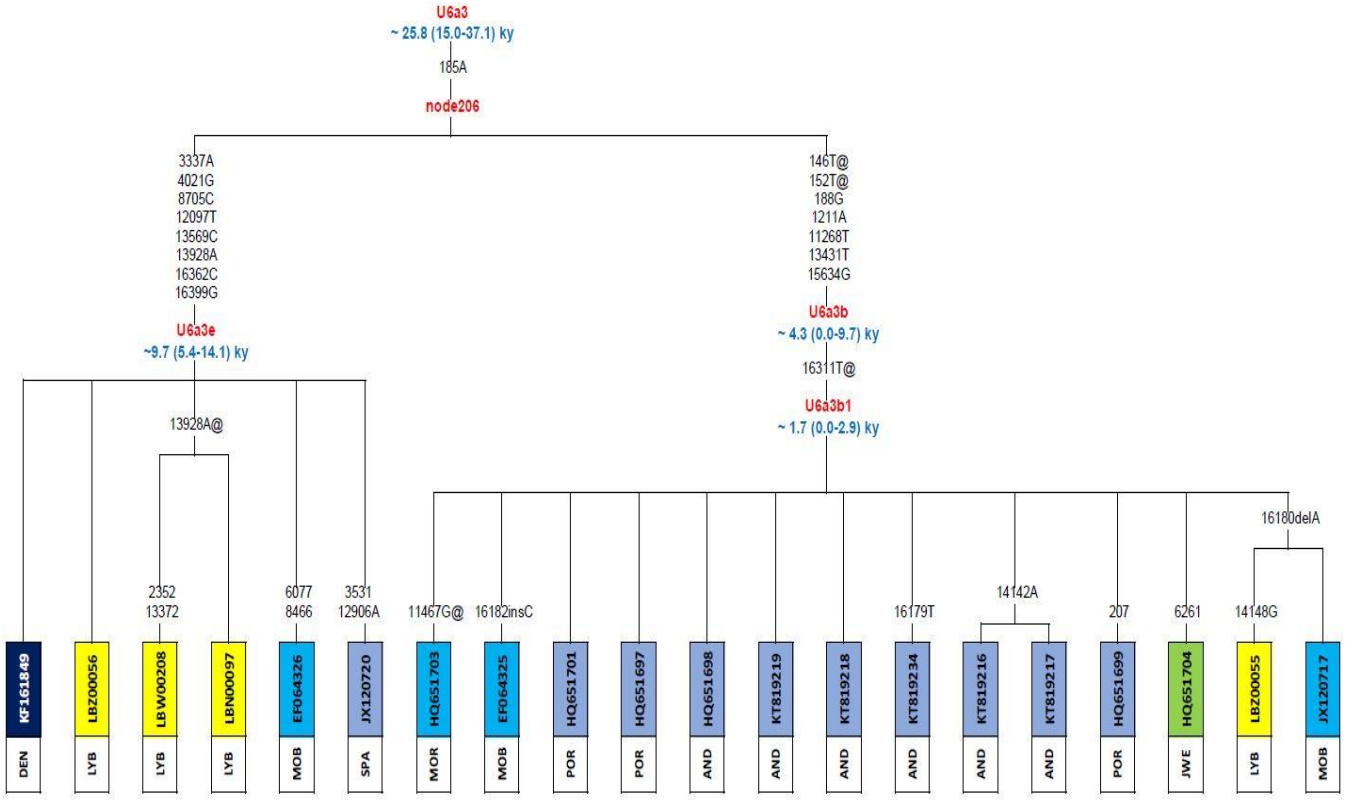

Figure 85. Tree of mtDNA sequences of U6a3 lineage

Libya in yellow, North Africa in sky blue, Near East in light green, Iberian in light blue (LYB, Libya; POR, Portugal; JEW, Jewish; MOR, MOB Morocco; SPA, Spain; DEN, Denmark; AND, Andalusia)

4.4.1.2.Sub-haplogroup U6b

U6b is defined by transitions at 16172, 16219 and 16311 dated to 12.5 kya (Pennarun et al., 2012) and also 12.5 kya in this study, It has more limited distribution to the west of North Africa, and north of the Iberian. U6b is further divided into U6b1, U6b3, U6b4, U6b3 dated to 3.9 kya (Secher et al., 2014) and 6 kya in this study.

Within U6b3, a new sub-group U6b3a is characterised by a transition at 235 in West Africa. All U6b Libyan samples from the Ghadames population occurred in the U6b3a with Moroccan and Tunisian subjects. This branch was dated in this study to 1.1 kya (Figure 86).

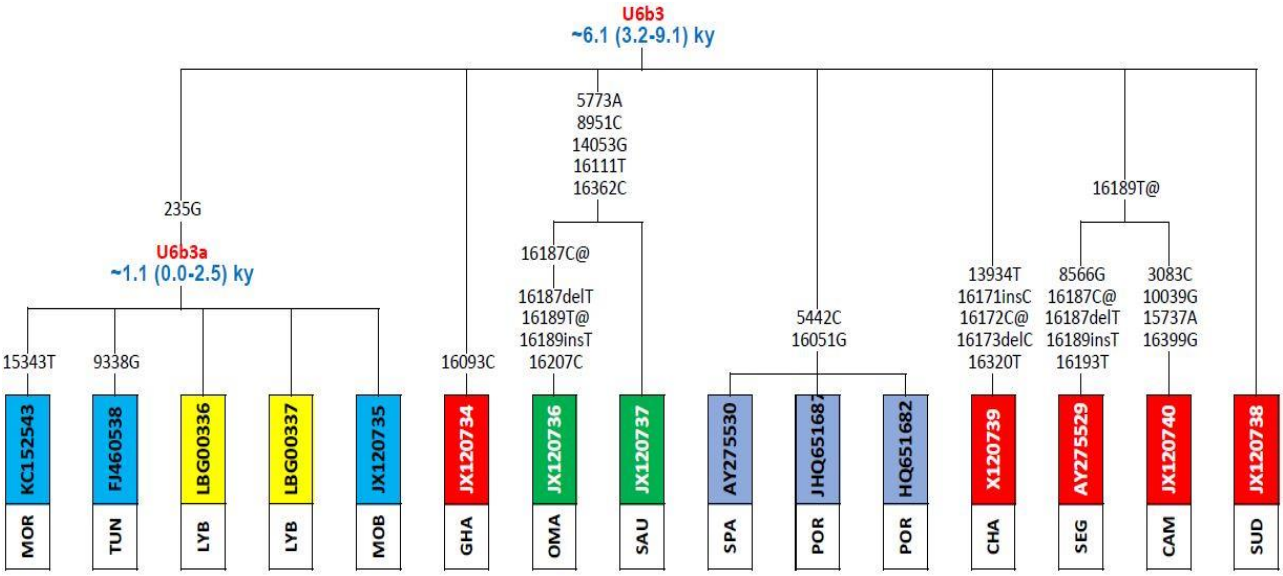

Figure 86. Tree of complete mtDNA sequences of U6b3 lineage

Libya in yellow, North Africa in sky blue, Africa in red, Iberian in light blue, Arabia in green (LYB, Libya; POR, Portugal; OMA, Oman; MOR, MOB Morocco; SPA, Spain; Tun, Tunisia; SAU, Saudi; CHA, Chad; SEG, Senegal; CAM, Cameron; SUD, Sudan; GHA, Ghana)

Founder analysis was done for Haplogroup U6, Figure 87, assuming that North Africa and Europe are the sink population and Near East, Asia and East Africa as a source population for this haplogroup. The result showed three peaks or dispersal times; the first migration time was at around 15 kya associated with Iberomaurusian culture in North Africa. The spread may be related to the earliest sub-clades of U6 such as U6a and U6a1. The second episode of U6 migration was 8.2 kya during the Capsian culture that may have lead to the expansion of U6b and U6a8. The recent migration at 1.2 kya may associate with Arab movement spreading the Islam and Arab language to North Africa (Figure 87).

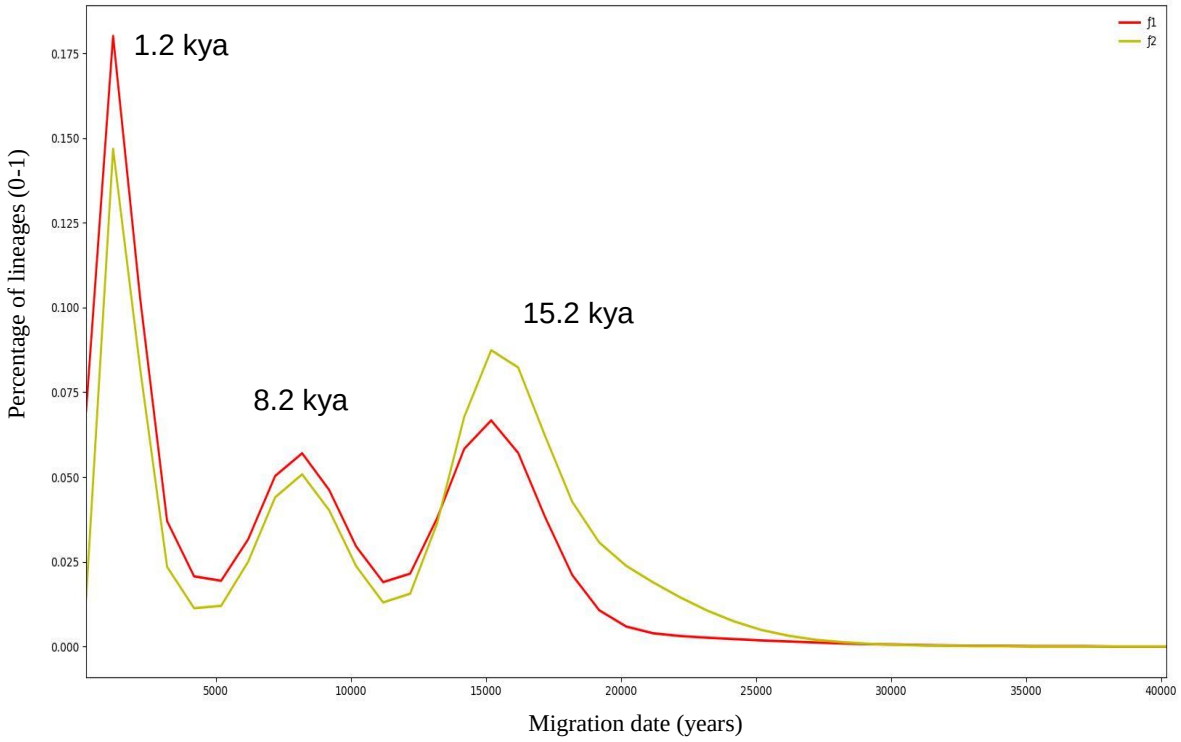

Figure 87. Founder distribution of U6 lineages across migration times

4.2.2. Haplogroup M1

Haplogroup M is found primarily in India, and other eastern region and was probably part of the original out of Africa event around 60 kya (Kivisild et al., 2004; Macaulay et al., 2005; Rando et al., 1998; Tanaka et al., 2004; Sun et al., 2006). Haplogroup M1, however, has been known as an African origin haplogroup, even though the all other subclades of haplogroup M are found in South Asia, East Asia, and Australasia (Watson et al., 1997). M1 is a sister haplogroup to Haplogroup D, dated to 29 kya (Winters, 2010), is also one of the indigenous North African clusters which are more abundant in Eastern Africa/ Ethiopia, Sudan where most of the unique sequences are found and Saudi Arabia (Pennarun et al., 2012). It is unclear whether M1 sequence type in the Near East arrived recently from Africa or an Asian origin with back-migration to Africa being possible (González et al., 2007) Figure 88 illustrates the diversity of M1 haplogroup.

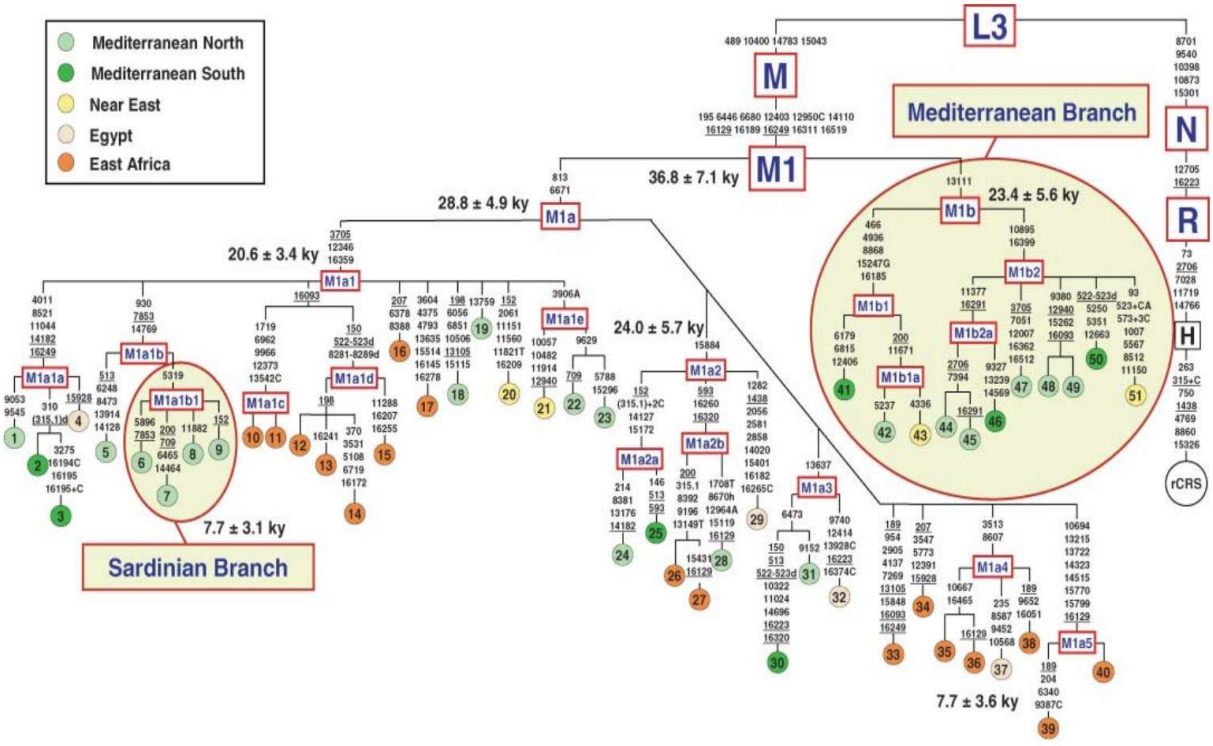

Figure 88. Tree of mtDNA sequences of haplogroup M1

Taken from (Olivieri et al., 2006)

4.2.2.1. Subhaplogroup M1a

M1a has close coalescent ages around the LGM ~21 kya (Pennarun et al., 2012). Among M1a the most diverse clade is M1a1 which is defined by mutations at 3705, 12346 and 16359 and found in the Mediterranean and East Africa (Olivieri et al., 2006). M1a3 is defined by two transitions at 13637 and 16223 date to 11.7 kya (Pennarun et al., 2012). Libyan samples in this clade were found in branch M1a3b1 that is defined by transitions at 1503, and 16129 that are also found in Moroccan, Spain and Russian samples (Figure 89).

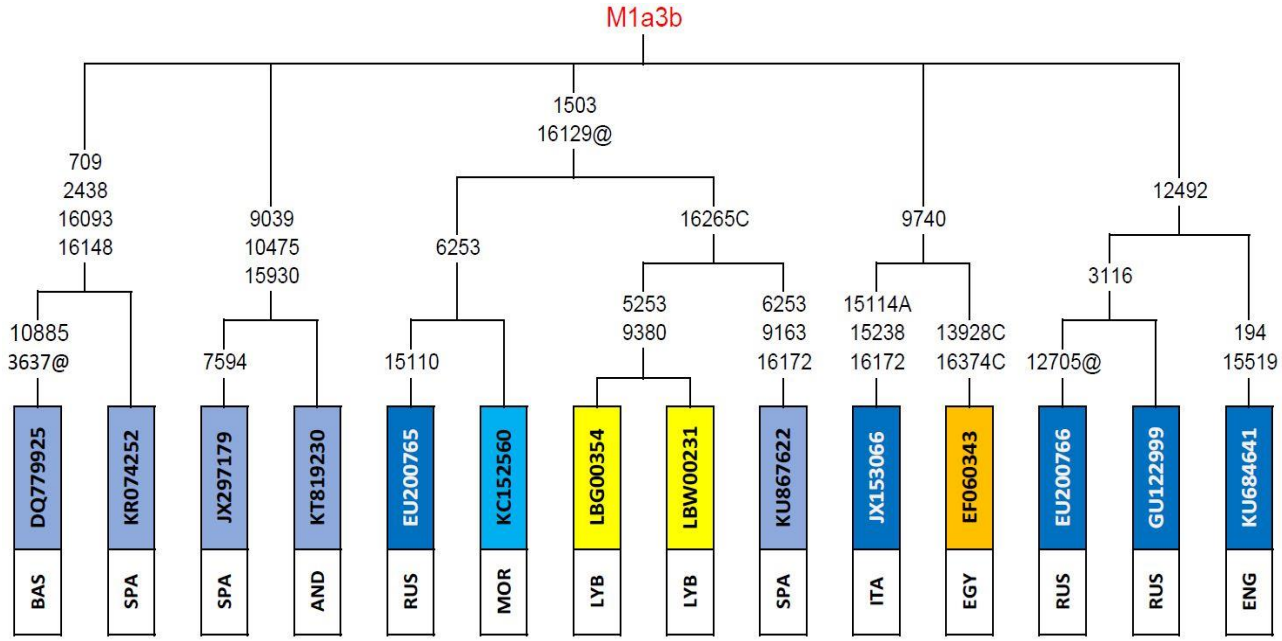

Figure 89. Tree of complete mtDNA sequences of M1a3b lineages

Libya in yellow, North Africa in sky blue, Iberian in light blue, Egypt in orang Europe in Blue (LYB, Libya; BAS, Basque; AND, Andalusia; MOR, MOB Morocco; SPA, Spain; RUS, Russia; ITA, Italy; EGY, Egypt; ENG, England)

4.2.2.2. Subhaplogroup M1b

M1b has been dated to 20 kya around the LGM period (Pennarun et al., 2012). Sub-haplogroup M1b1 is defined by the mutation 16185 and is mainly present in both sides of the Mediterranean (Olivieri et al., 2006); however, it is also found in Arabia and East Africa. M1b1 has been dated to ~9.8 ~10 kya (Pennarun et al., 2012). Subclade M1b1 was also present in Zuwara Berber, located in clade M1b1a that also contains samples from the Mediterranean, Levant and Arabia (Figure 90).

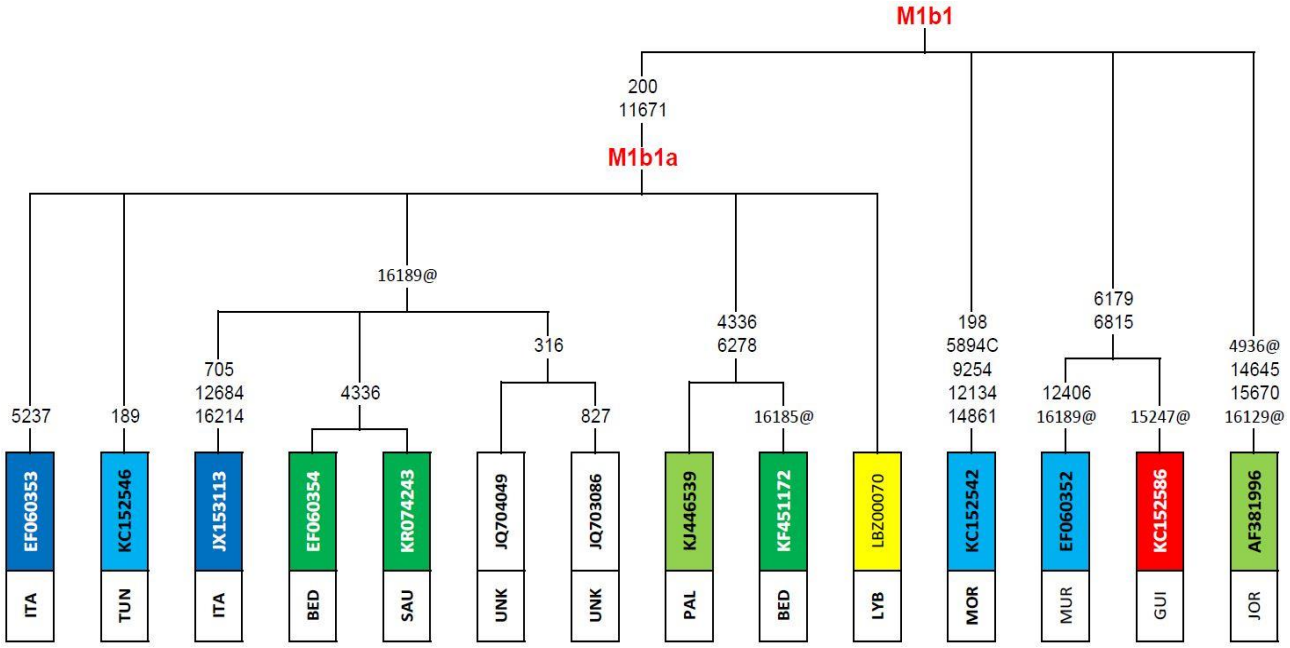

Figure 90. Tree of complete mtDNA sequences of M1b1 lineage

Libya in yellow, North Africa in sky blue, Arabia in green, Europe in Blue, Africa in red, Near East in light green (LYB, Libya; TUN, Tunisia; Bed, Bedouin; MOR, Morocco; MUR, Mauritania; SAU, Saudia; ITA, Italy; JOR, Jordan; GUI. Guinea; PAL, Palestine)

M1b2 clade was also present in Libyan subjects from African Libyan and Berber group, in a branch dated to 14 kya (Pennarun et al., 2012). Both Libyan samples shared a branch with a sample from a Moroccan Berber (Figure 91).

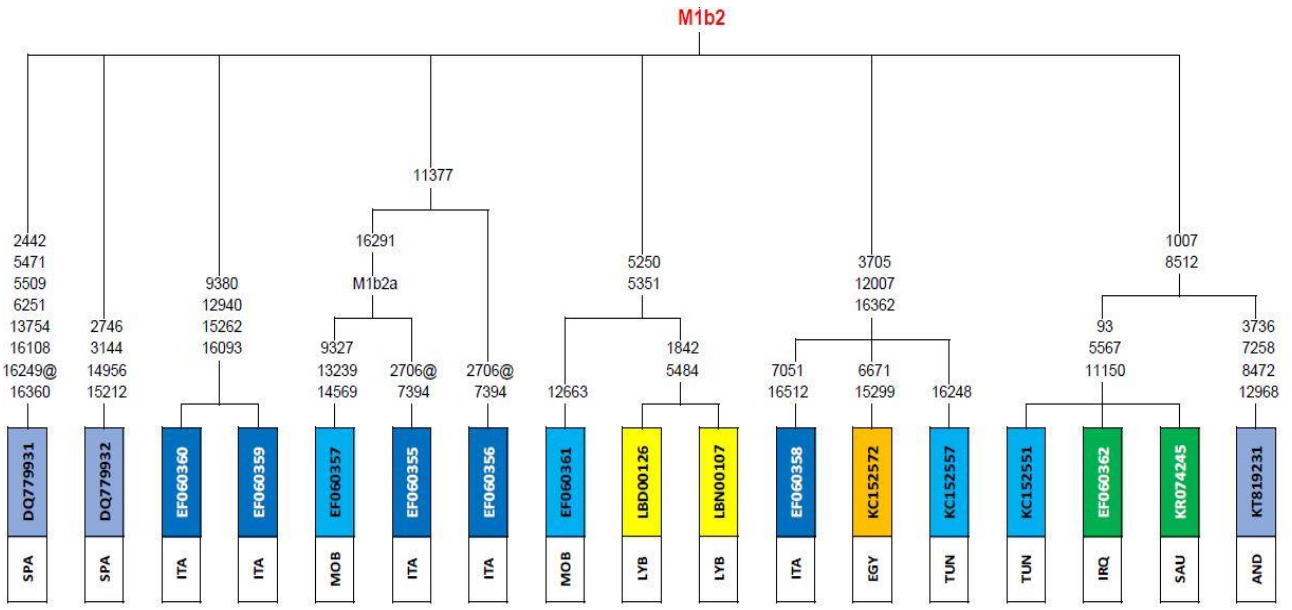

Figure 91. Tree of complete mtDNA sequences of M1b2 lineages

Libya in yellow, North Africa in sky blue, Arabia in green, Europe in Blue, Arabia in green, Iberian in light blue (LYB, Libya; IRQ, Iraq; MOB, Morocco; TUN, Tunisia; SAU, Saudia; ITA, Italy; SPA, Spain; EGY, Egypt; AND, Andalusia)

From the analysis of Haplogroup U6 and M1 and comparing the coalescent time, it is clear that these lineages have been present in North Africa since Middle Palaeolithic or at least Upper Palaeolithic with the expansion of the Iberomaurusian industry. It has been suggested that populations of haplogroups U5, U6 and M1 were living separately in south-western Asia and they were affected by the same event. While U5 groups colonised Europe, MI and U6 groups migrated toward North Africa, either directly through the Mediterranean or after a period of settlement in the Levant refuge to reach North Africa in Upper Palaeolithic or early Mesolithic (Olivieri et al., 2006). Like U6 haplogroup M1 was mainly found among Berber populations in Libya. While a Zuwara Berber sample occurs in the clade M1b1 which also contains samples from different regions including Middle East, North Africa, Europe and Africa, the Western Libyan samples were located in M1a3b clade which only found in Iberia and North Africa. Finally, a Nafusa Berber sample was located in the M1be clade shared with Moroccan Berber.

4.3. Sub-Saharan Lineages in the Libyan population.

Sub-Saharan lineage L is the mitochondrial DNA root of the human maternal phylogenetic tree. It represents the most ancestral mtDNA lineage of all present living modern humans. It is believed to suggest an ultimate African origin of modern human's dates to 173, 155 and 193 kya by Mishmar, Kivisilid and Soares respectively (Soares et al., 2009, Mishmar et al., 2003, Kivisild et al., 2006).

Its major sub-haplogroups include L0, L1, L2, L3, L4, L5 and L6, with all non-Africans exclusively descended from just haplogroup L3. It is known that sub-Saharan lineages in North Africa represent from 25% to 50% of North African mtDNA pool. The frequencies of these lineages increase as geographic proximity to sub-Saharan Africa increases. The Sahara acts as a strong geographical barrier against gene flow between south and North of the Sahara, at least since the desertification affected a larger region 5 kya, and ended with humid and greening conditions that started by 10 kya, in the Holocene period (Harich et al., 2010).

Analysis of the samples in this study revealed of presence wide range of these lineages including L0, L1, L2, L3 and L4 (Figure 92) and indicated that Sub-Saharan lineages in Libya represented 30% of the Libyan population reaching 75% among dark-skinned African Libyans.

4.3.1. Haplogroup L0

Haplogroup L0 is the earliest mtDNA tree branch in Africa that is parallel to the branch containing all other haplogroups, (L1-6) dated to 112 kya by (Kivisild et al., 2004) and by Soares to 141 kya. (Soares et al., 2009). L0 arose in East Africa and then divided into sub-haplogroups L0a, L0d, L0f, and L0k. While L0a, L0b and L0f is found in East and Central/West Africa, L0d and L0k are specific to South African (Salas et al., 2002). L0 is represented in the Libyan samples primarily by clade L0a and an L0f and was mainly seen among African Libyans.

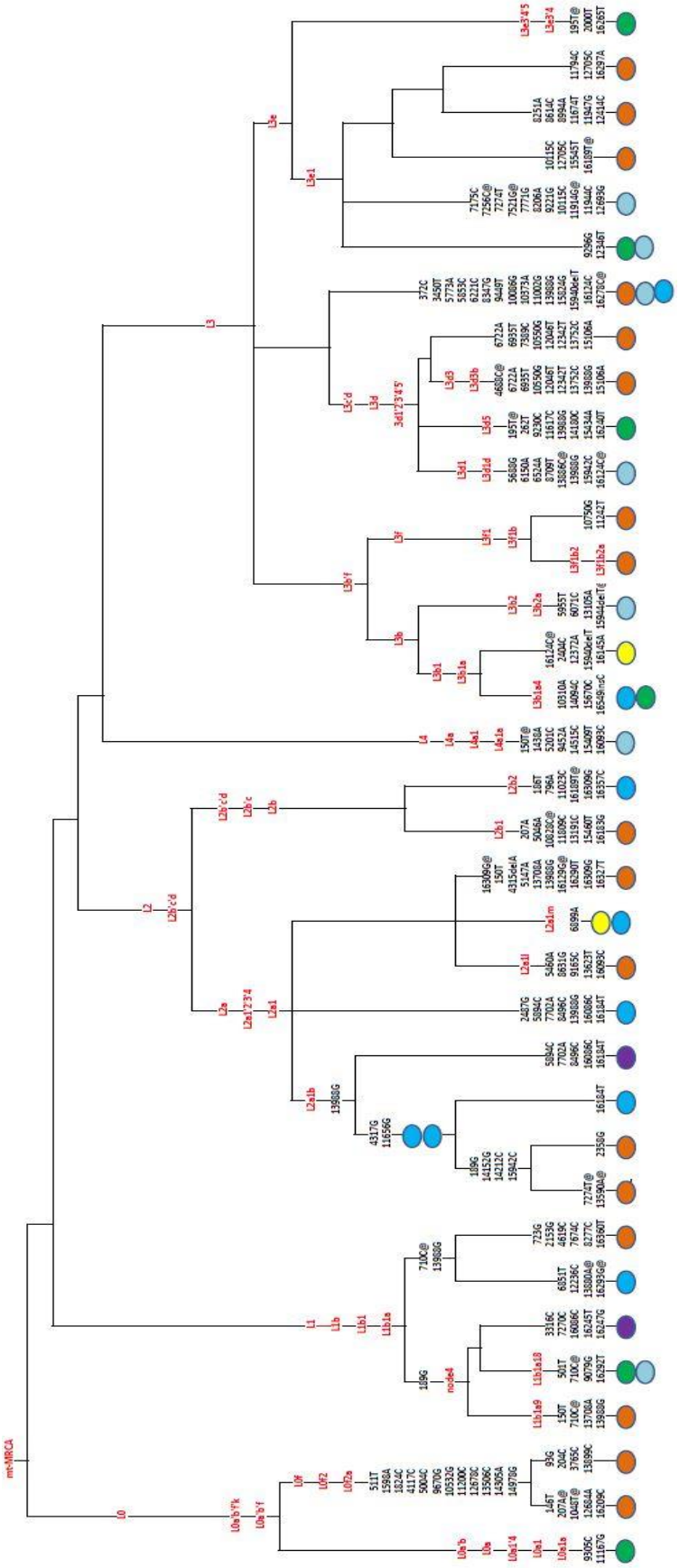

Figure 92. The tree of complete mtDNA of sub-Saharan lineages in Libya

Western Libyan Arab samples in orange, Eastern Libyan Arab samples in light blue, Zouwara Berber in purple, Nafusa Berber in yellow, Ghadames population in blue and African Libyan in green.

4.3.1.1. L0a sub-haplogroup

L0a diversified 40 kya in eastern Africa (Salas et al. 2002) where it reaches a frequency of up to 25% and is common in eastern, central, and southeastern Africa, but it is found at low frequency in North of the Sahara (Fendt et al., 2012). Haplotype L0a1 is most abundant L0a haplotypes in Africa and is divided into several subhaplogroups, L0a1a-L0a1d each with unique distribution among African regions. L0a1 counts for the majority of L0a1 and is widely distributed in East Africa and moderate frequency in northeast Africa (C˘erny et al. 2006). L0a1a which is found in Central and East African (Salas et al. 2002) also represents the majority of L0a lineages in Libya. The L0a1a clade is mainly found in African Libyans in this study and was previously dated to 11.8 kya (Soares et al., 2009) which shares a branch with a Chadic sample defined by transversion at 9305 (Figure 93). The presence of L0a has been attributed to the major recent migration of the sub-Saharan population, where the slave caravans came to the Southern region of Fezzan, in 1929, and then migrated to the coastal cities during the Second World War such as Zliten and Tagora (Wright, 2012) (Figure 93).

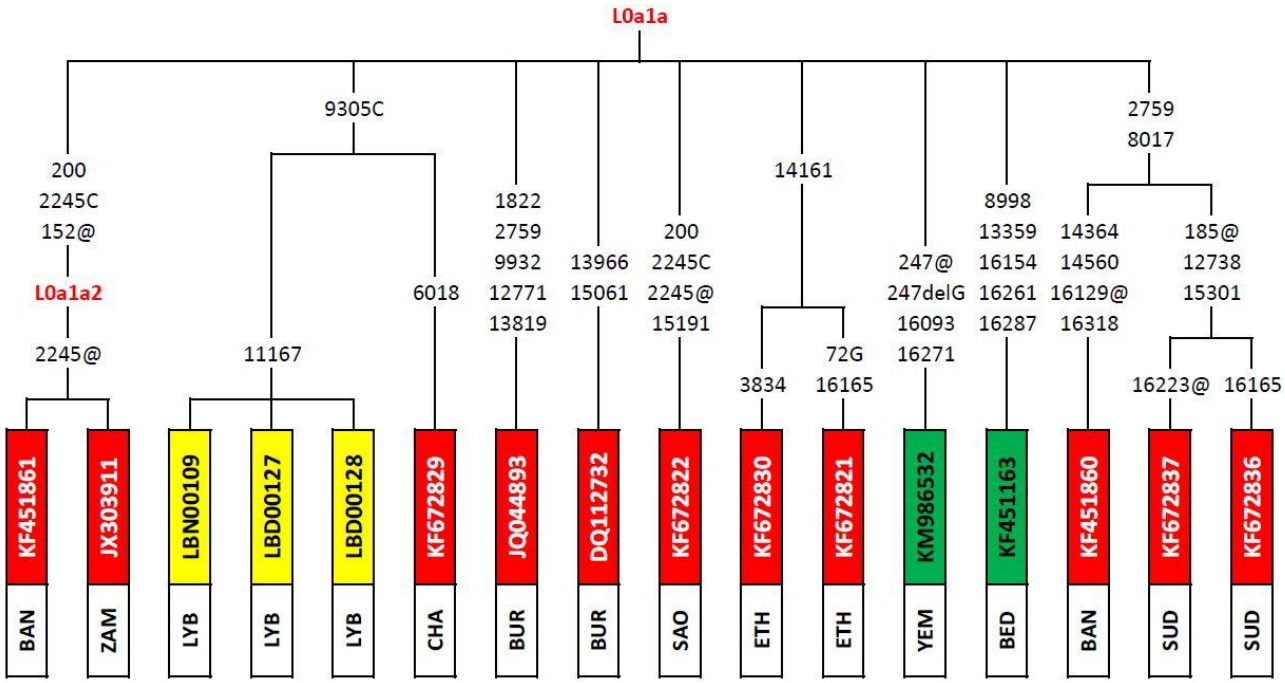

Figure 93. Tree of complete mtDNA sequences of L0a1a lineages

Libya in yellow, Arabia in green, Africa in Red (LYB, Libya; YEM, Yemen; BED, Bedouin; BAN, Bantu; ZAM, Zambia; CHA, Chad; BUR, Burkina; SAO, Saotome; ETH, Ethiopia; SUD, Sudan; UNK, Unknown)

4.3.1.2. Clade L0f

L0f is rare African clade mainly found among hunter-gatherer populations (Salas et al., 2002). Among L0f three clades were identified L0f, L0f2 and L0f3. The L0f2 sub-haplotype was found in northern Kenya**,** dated by Behar to 56.8 kya (Behar et al., 2012b). Libyan samples in this clade were restricted to Arabic samples classified into a branch named L0f2a Figure 94, dated to 45 kya (Behar et al., 2012b). The majority of samples found in this tree are east African, and as a general trend of the Arabic population, most of their haplogroups are of the Eastern African region. The probable source of these Eastern African lineages were the Arab Muslims who migrated to North Africa in the seventh century.

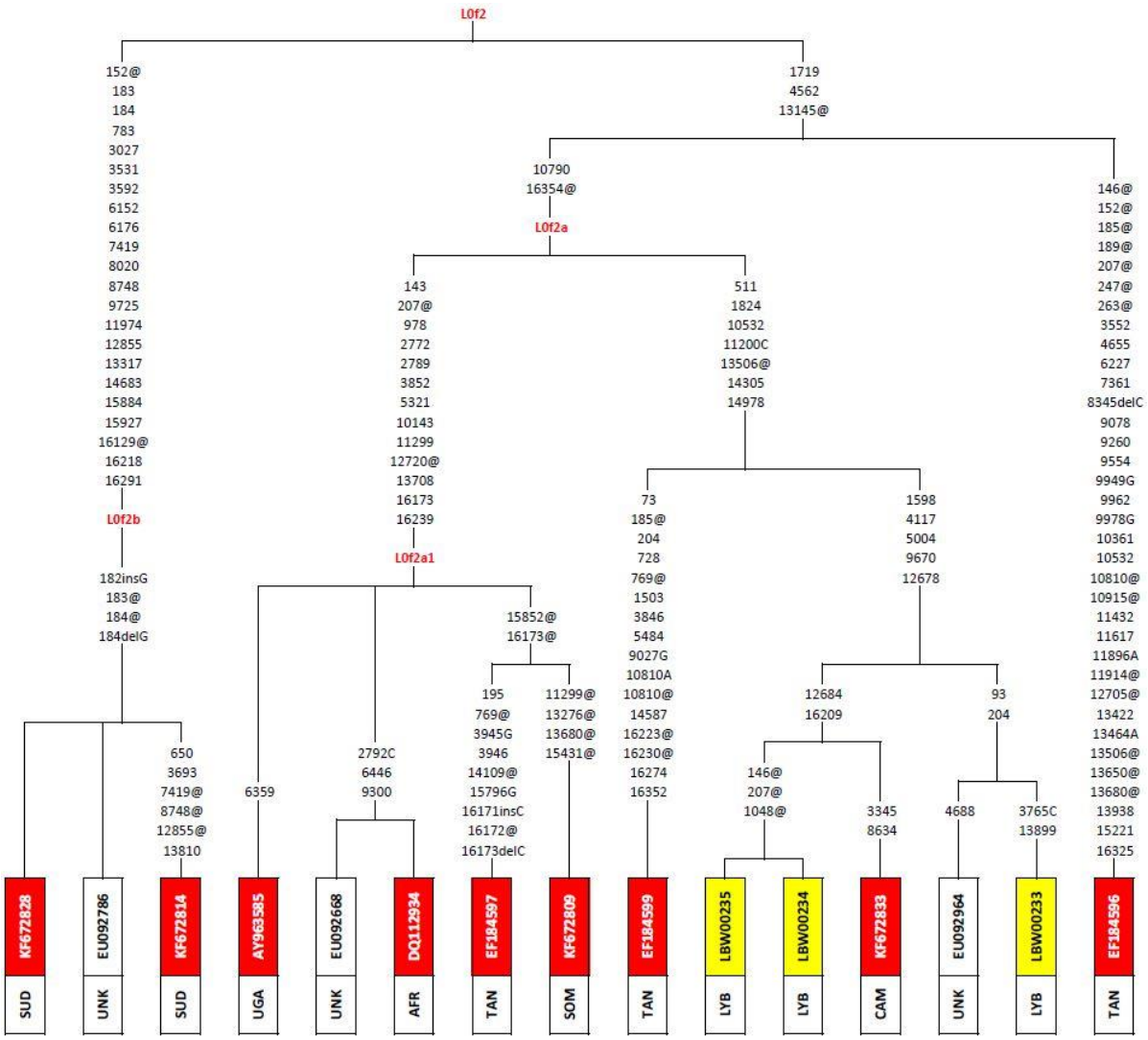

Figure 94. Tree of complete mtDNA sequences of L0f2a lineages

Libya in yellow, Africa in Red (LYB, Libya; UGA, Uganda; TAN, Tanzania; CAM, Cameron; Afr, Africa; SUD, Sudan; UNK, Unknown)

4.3.2. Haplogroup L1

L1 has a coalescence time of 123 kya (Soares et al., 2009) and is composed of L1b and L1c. L1c frequently occurs among Central African Bantu speakers and probably arose among peoples who live near the Atlantic coast in western equatorial Africa. L1b is concentrated in western Africa, but it also found in central and northern Africa. (Salas et al., 2002, Watson et al., 1997), dated to 9 kya (Soares et al., 2009) and 4.3kya (Behar et al., 2012b). The vast majority of L1 samples in Libya belonged L1b1a, represented by L1b1a9 and L1b1a18 clades which are mainly found among the Western population of Libya including African Libyans. L1c was also detected in one sample belonging to African Libyan (Table 18). The Libyan population in this haplogroup were located in branch constituted mostly by Western African populations and additionally a few African Americans (Figure 95).

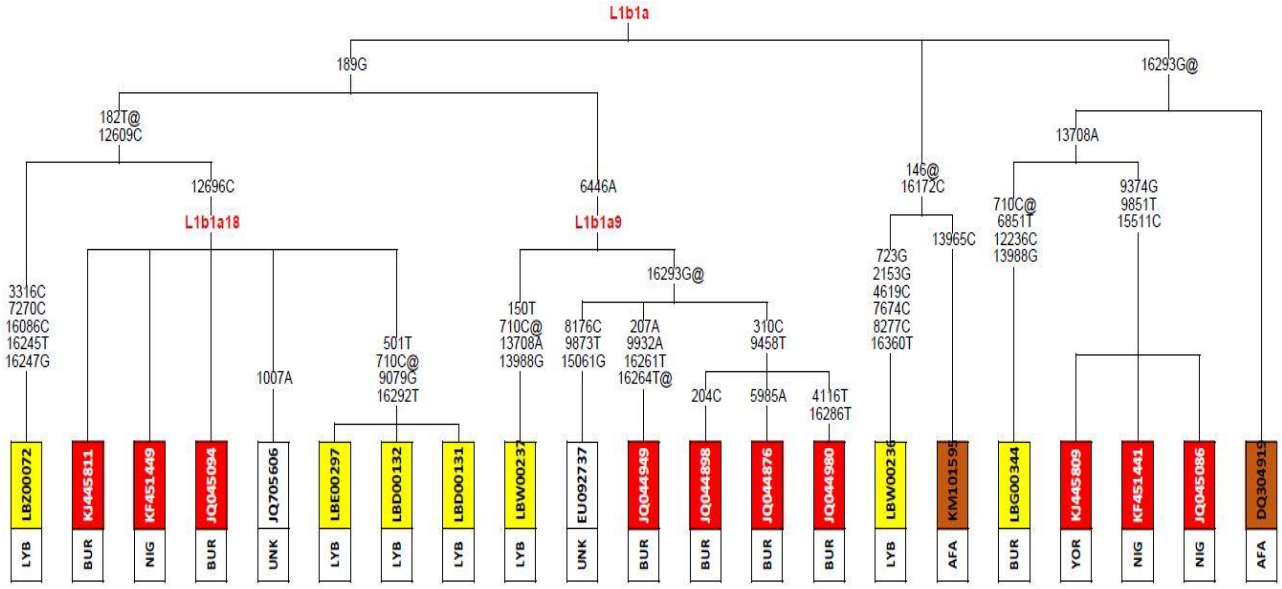

Figure 95. Tree of complete mtDNA sequences of L1b1a lineages

Libya in yellow, USA in brown, Africa in Red (LYB, Libya; BUR, Burkina; NIG, Nigeria; YOR. Yoruba; AFA, African American)

Analysis of L1b haplogroup among the sub-Sahara population showed a horizontal gradient distribution between West and East regions. (Harich et al., 2010). These lineages were most likely

introduced in Libya and North Africa during the early Holocene after a humid period (Hernandez et al., 2015).

4.3.3. L2 haplogroup

Despite the haplogroup L2 accounting 30% of sub-Saharan lineages in Africa (Torrini et al., 2001) and high estimated coalescence time of 89.3 kya (Soares et al., 2009) it did not take part in human expansion out of Africa. Among L2 lineages, L2a splits at 48.3 kya and is the most frequent and widely geographically spread lineage, while L2b, L2c and L2d are dated to 48 kya, 14 and 17 kya respectively, and are concentrated in the West coast between Senegal and Mauritania Figures 96, 97. All Libyan L2 lineages were from the two subclades L2a1 and L2b.

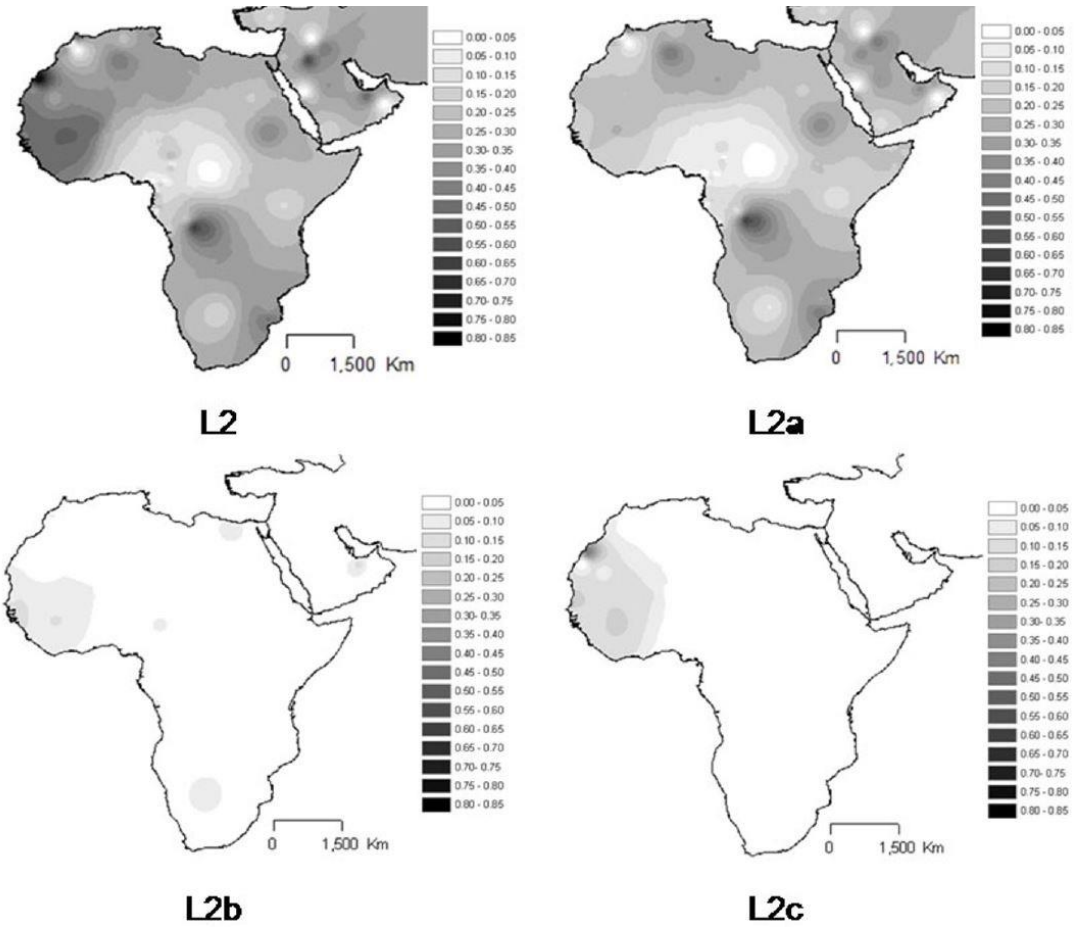

Figure 96. Map show frequency L2 haplogroup lineages in Africa and Arabia

Taken from (Harich et al., 2010)

4.3.3.1.Sub-haplogroup L2a1

L2a1 lineages have unique control region mutations that are diagnostic for distinguishing them, they have been dated to 21.5 kya (Soares et al., 2009) and 26.5 kya (Silva et al., 2015) and are distributed in the whole of Africa and spread to other continents, such as a European clade L2a1k3 and L2a1l2a which is connected to Ashkenazi Jews (Silva et al., 2015). The highest frequency of L2a lineages is in the Central African Baggara population (24%), an Afro-Asiatic speaking group that trace their ancestry to the Near East.

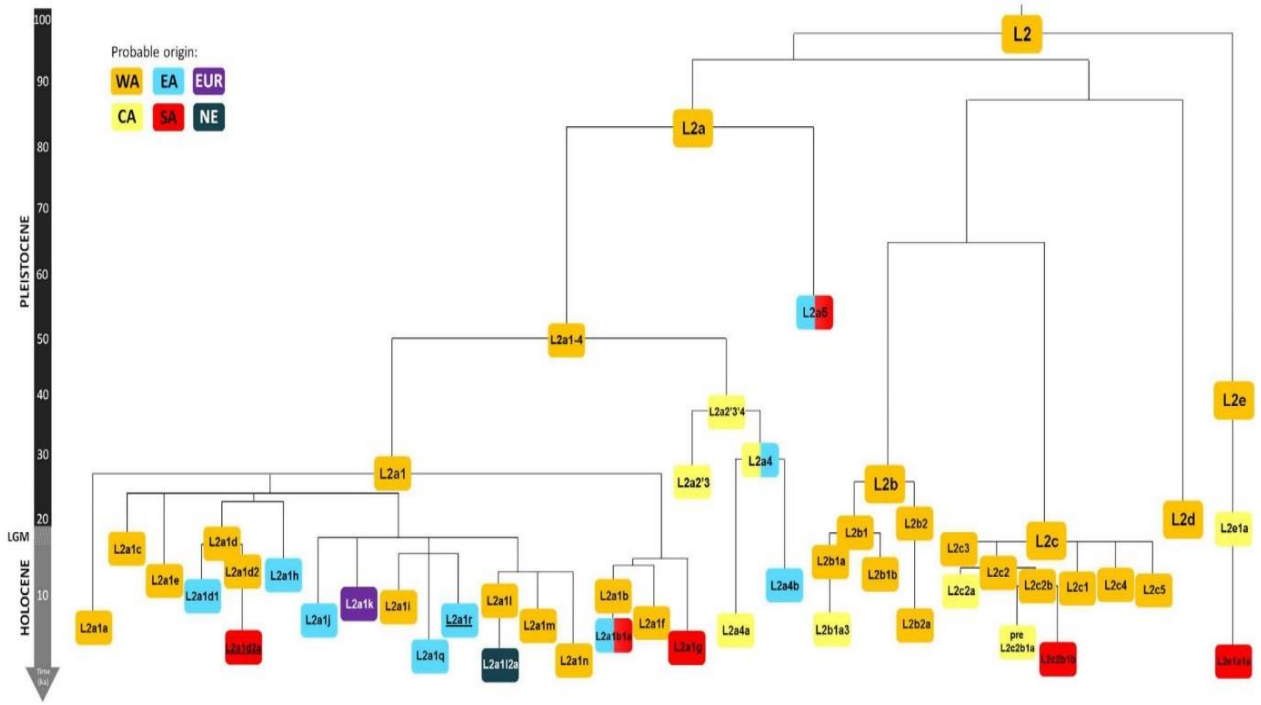

Figure 97. Phylogeny of maternal haplogroup L2

The age-based on Maximum likelihood, WA, Western Africa; CA, Central Africa; EA, Eastern Africa; SA, Southern Africa; EUR, Europe; NE, Near East/Arabian Peninsula Taken from (Silva et al., 2015)

L2a1 individuals genotyped in this study in the Libyan population belong to three West African clades: L2a1b, L2a1m and L2a1l (Figure 98).

4.3.3.2. L2a1b clade

L2a1b defined by 16189, 16192 and 10143 originated 11.4 kya in central Africa (Silva et al., 2015). L2a1b is present in southeastern Africans and is characterised by a transition at. 16192 (Salas et al., 2002). This L2a1b branch was dated to 16.2 kya years in this study. Libyan samples in this clade were found in a branch defined by transitions at 13988, 4317 and 11656, belonging to western Libya. The majority of the samples in this branch are western African and African American (Figure 98).

It has been suggested that there was an early Holocene introduction of these lineages in Libya and North Africa (Soares et al., 2012: Hernandez et al., 2015). The ages for the L2a1b in the Libyan population in this study was estimated at 15.6 kya, so the introduction of this clade was probably associated with an Iberomaurusian period or at least in early Holocene.

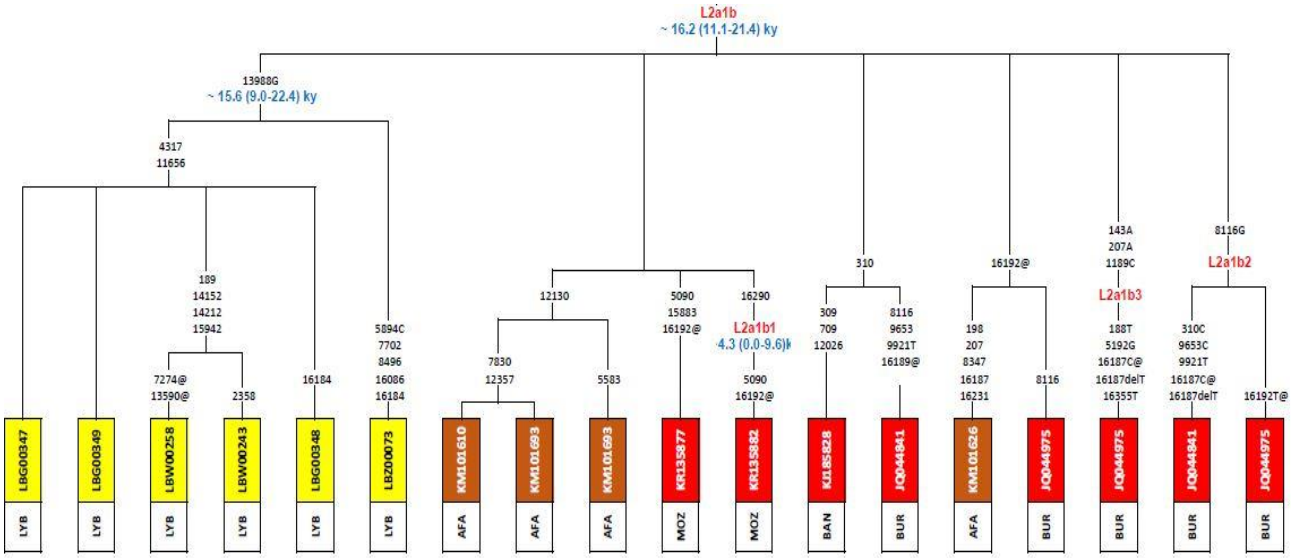

Figure 98. The tree of complete mtDNA sequences of L2a1b lineages

Libya in yellow, USA in brown, Africa in Red (LYB, Libya; BUR, Burkina; MOZ, Mozambique; AFA, African American)

The other L2a1 sequences found in this study were classified within L2a1l and L2a1m Figure 99. The small clade L2a1m was dated to 6.5 kya is primarily found only in Western/Central Africa, and L2a1l was dated to 11 kya showing a similar pattern in sub-Saharan Africa (Silva et al., 2015).

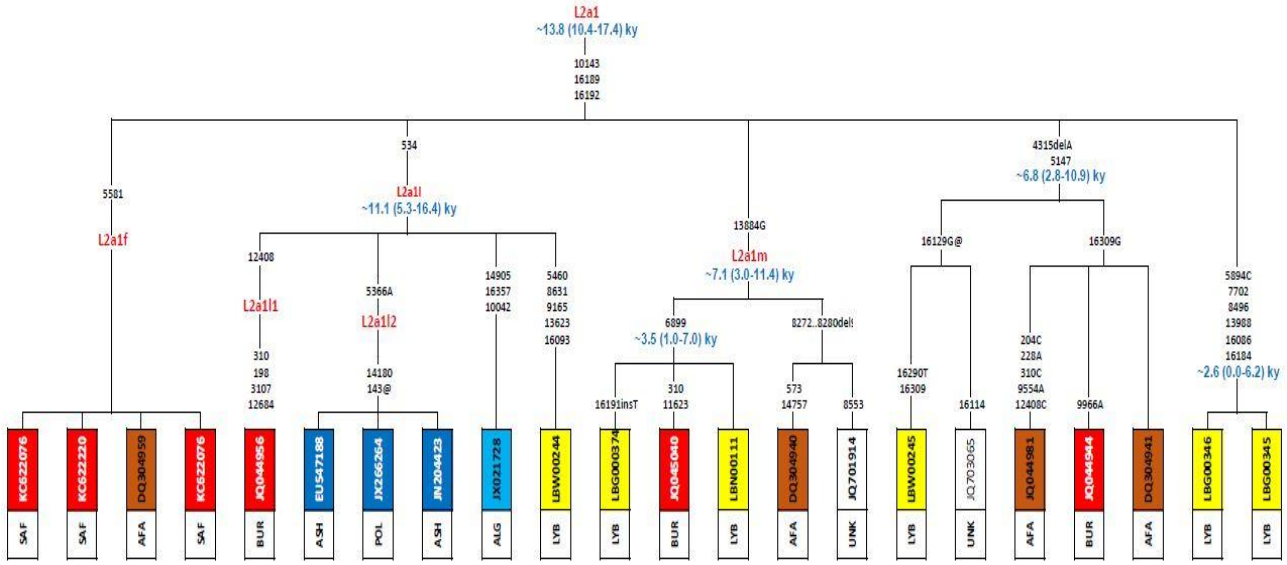

Figure 99. Tree of complete mtDNA sequences of L2a1 lineages

Libya in yellow, North Africa in sky blue, USA in brown, Africa in Red, Europe in blue (LYB, Libya; ALG, Algeria; ASH, Askinazi Jewish; POL, Poland, BUR, Burkina; SAF, South Africa; AFA, African American; UNK, Unknown)

4.3.3.3. Haplogroup L2b

L2b arose ~26.0 kya and is divided into four branches that originated in Western/Central Africa showing a high frequency in the west coast especially between in Senegal and Mauritania. This clade is represented in Libya by two samples, one classified as L2b1 and the other L2b2 (Figure 100).

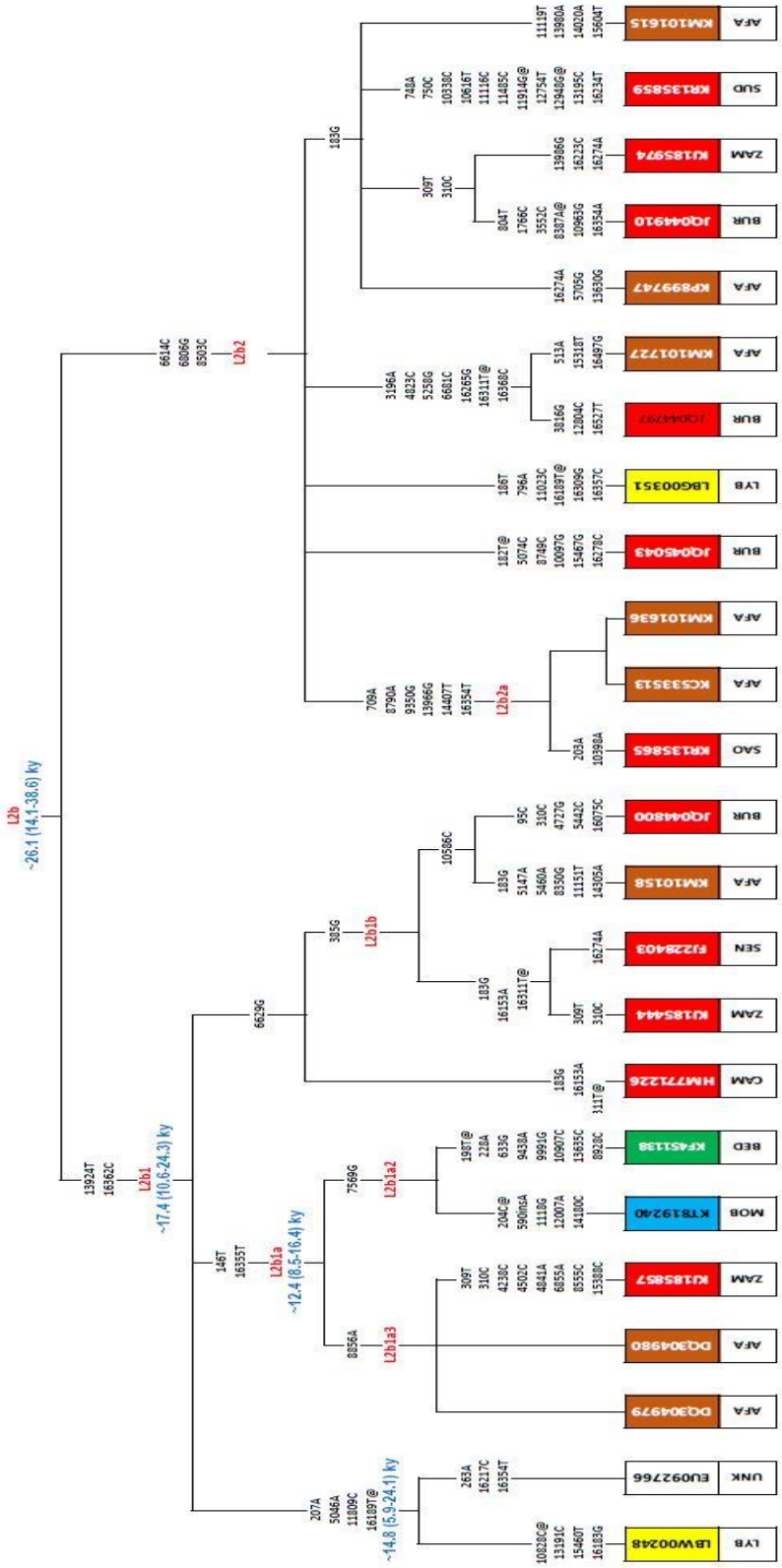

Figure 100. Tree of complete mtDNA sequences of L2b lineages

Libya in yellow, North Africa in sky blue, USA in brown, Africa in Red (LYB, Libya; MOB, Moroccan Berber; BED, Bedouin;CAM, Cameron; ZAM, Zambia; SEN, Senegal; SAO, Saotome; BUR, Burkina; SAF, South Africa; AFA, African American; UNK, Unknown)

Founder analysis was performed assuming sub-Saharan Africa as a source population and Libya and North Africa as a sink (Figure 101) The graph shows a peak at 19 kya with *f2* and *f1*. It also shows a major peak was towards the present that may correspond to the recent trans-Saharan slave migration in the early nineteenth centuries (Harich et al. 2010).

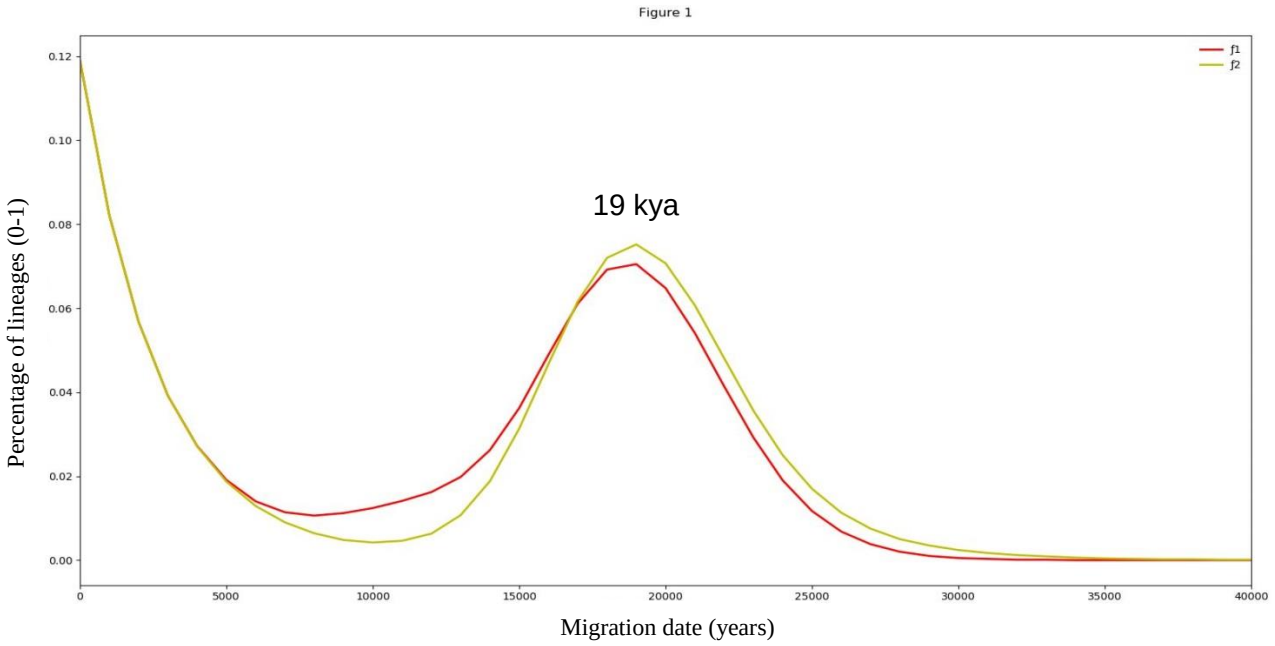

Figure 101. Founder distribution L2 lineages across migration time

4.3.4. Haplogroup L3

The L3 haplogroup is distributed throughout the African continent; however, it is found at the highest frequencies in the Eastern part of the continent, where it is about 50% of all types. This frequency profile suggests an origin for L3 in East Africa (Torroni et al. 2006). This idea is also supported by the fact that the out-of-Africa migration, took place from a source in East Africa 60 kya and gave rise only to L3 lineages outside Africa (Watson et al., 1997). It seems likely that L3 dates somewhere between 60 and 70 kya (Figure 102) (Soares et al., 2012).

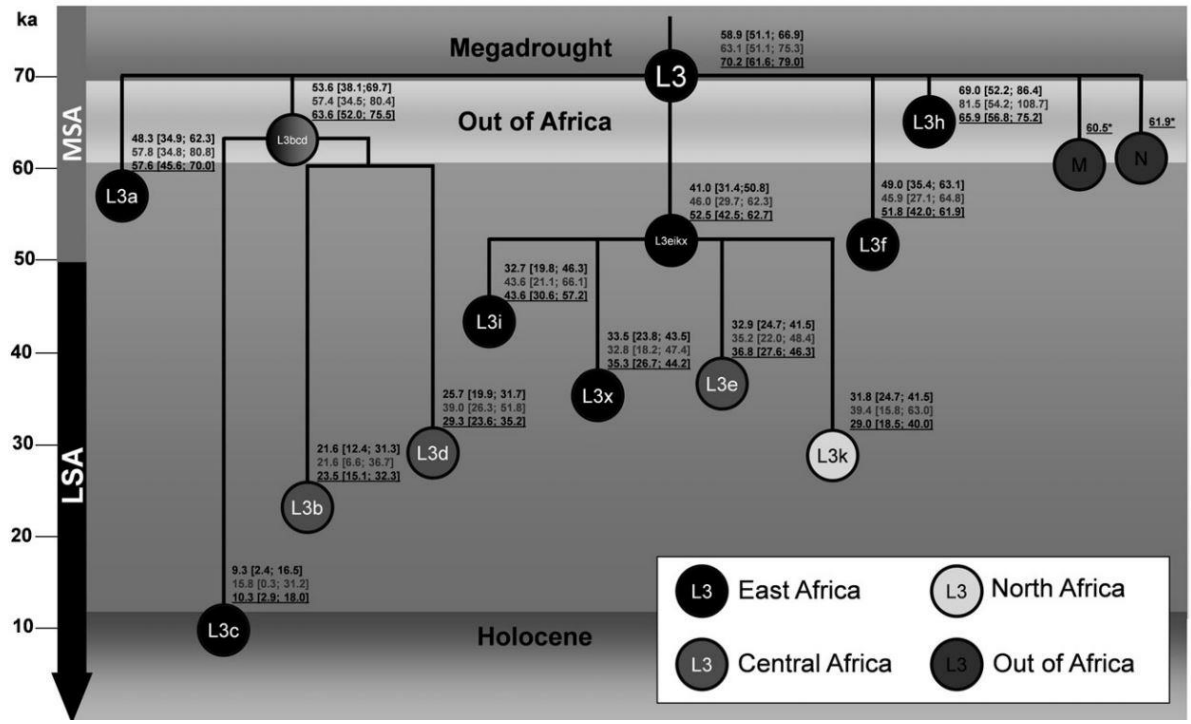

Figure 102. Tree of haplogroup L3
Taken from Soares et al., 2012

4.3.4.1. Subhaplogroup L3f

Lf3 is the most widely distributed and most diverse sub-haplogroup in East Africa that branched off the main L3 haplogroup in eastern Africa between 40 kya to 50 kya (Soares et al., 2012) and showed the highest frequency among Afro-Asiatic speaking people in Northeast Sudan 27%; however, it shows some dispersal other parts of Africa continent. The haplogroup has also crossed the Red Sea to the Arabian Peninsula and other parts of the Near East (Watson et al., 1997). The subclade had spread at an early date to West Africa about 20 kya before the peak of the Ice Age when the Sahara desert expanded, and much of the northern part of the continent was uninhabitable (Salas et al., 2002). L3f1 is divided into two main subclades (L3f1a and L3f1b) with basal lineages from Eastern Africa (Soares et al., 2012).

Libyan samples in this haplogroup were found in a branch named L3f1b dated in this study to 9.9 kya, constituted by sequences mainly from Eastern Africa. It also contains samples from Iran and

Egypt (Figure 103). One of the Libyan sequences occurred in branch defined by 16172 and 16266 transitions named as L3f1b2a contained an Egyptian sample dated to 3.2 kya. The other Libyan sequence occurs within the same branch as Chadic and Sudanese population samples.

The possible explanation of the presence of this lineages is due to the direct connection to western Africa through Chad basin or through other North African populations which were in turn connected with other parts of western Africa (Watson et al., 1997).

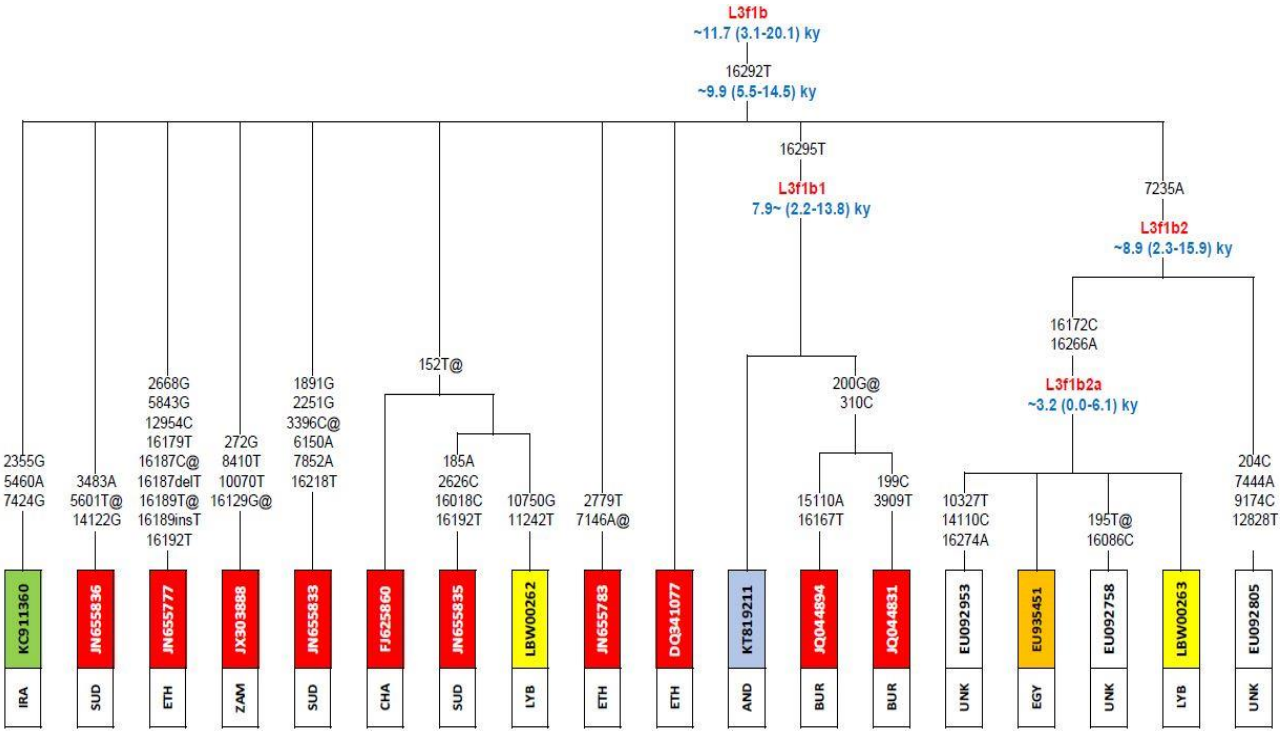

Figure 103. Tree of complete mtDNA sequences of L3f1b lineages.

Libya in yellow, Africa in Red, Spain in light blue, Egypt in orange, Iran in light green (LYB, Libya; EGY, Egypt; AND, Andalusia; IRA, Iran; SUD, Sudan; ZAM, Zambia; ETH, Ethiopia; CHA, Chad; BUR, Burkina; UNK, Unknown)

4.3.4.2. Subhaplogroup L3e

L3e sub-haplogroup is the most widespread and ancient of the African L3 clades, representing a third of all L3 haplogroup in sub-Saharan Africa (Soares et al., 2012). It has been suggested that the origin of this haplogroup is in the Central Africa/Sudan (Salas et al., 2002) and Central Africa (Soares et al., 2012). This lineage was found in African Libyans at a frequency of 12.5%, forming a

branch defined by 193 and 13708 translations (Figure 104). The presence of L3e lineage among African Libyan is probably due to the major recent migration of the sub-Saharan population, where the slave caravans came to the Southern region, Fezzan, in 1929, and then migrated to the coastal cities during the Second World War such as Zliten and Tagora.

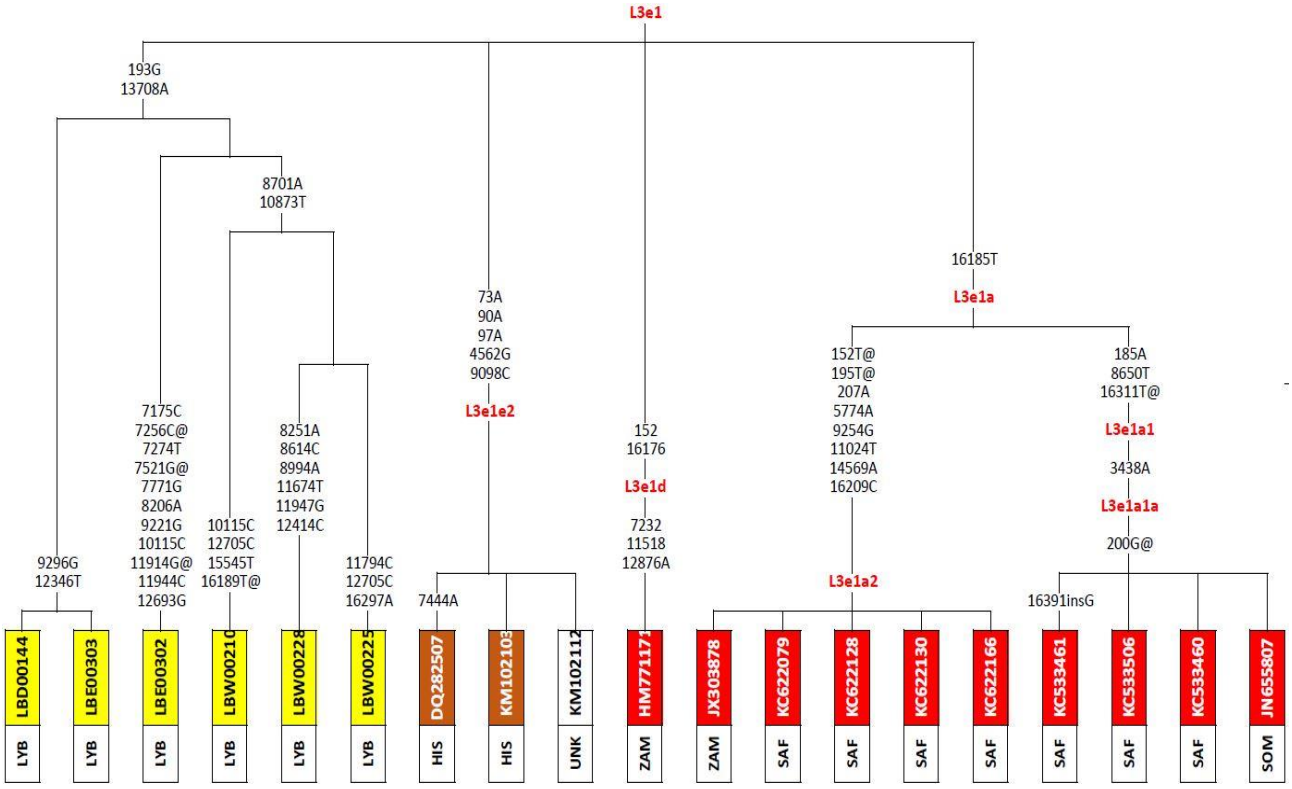

Figure 104. Tree of complete mtDNA sequences of L3e1 lineages

Libya in yellow, USA in brown, Africa in Red (LYB, Libya; HIS, Hispanic; ZAM, Zambia; SAF, South Africa; AFA, African American; SOM, Somalia; UNK, Unknown)

4.3.4.3. Subhaplogroup L3b

L3b is the lineage that represents the earliest dispersal lineages within L3 in Africa (Soares et al., 2012). L3b has a mainly western African distribution that is found more frequent in Northwest African samples and rare in North East African populations (Salas et al., 2002). L3b was dated in this study to 25.5 kya. The majority of Libyan sequences in this clade are African Libyan in addition to Saharan Berbers from Ghadames, found in different branches (Figure 15). The Ghadames population samples are located in branch defined by 1710 and 9605 transitions named

L3b1a4 that also contains a Tunisian sample dated in this study to 7.9 kya, while the rest of sequences are located in branch defined by nucleotide substitutions at 372, 8347, 15311 and 16362 dated in this study to 5.7 kya. These lineages were found at the percentage of 5.24% mainly in African groups suggesting that their origin in Libya might be due to the impact of the trans-Saharan slave trade routes that were established during recent times (Figure 105). The significant gradient of frequencies of haplogroups L3b agrees with this sub-Saharan genetic exchange within North Africa.

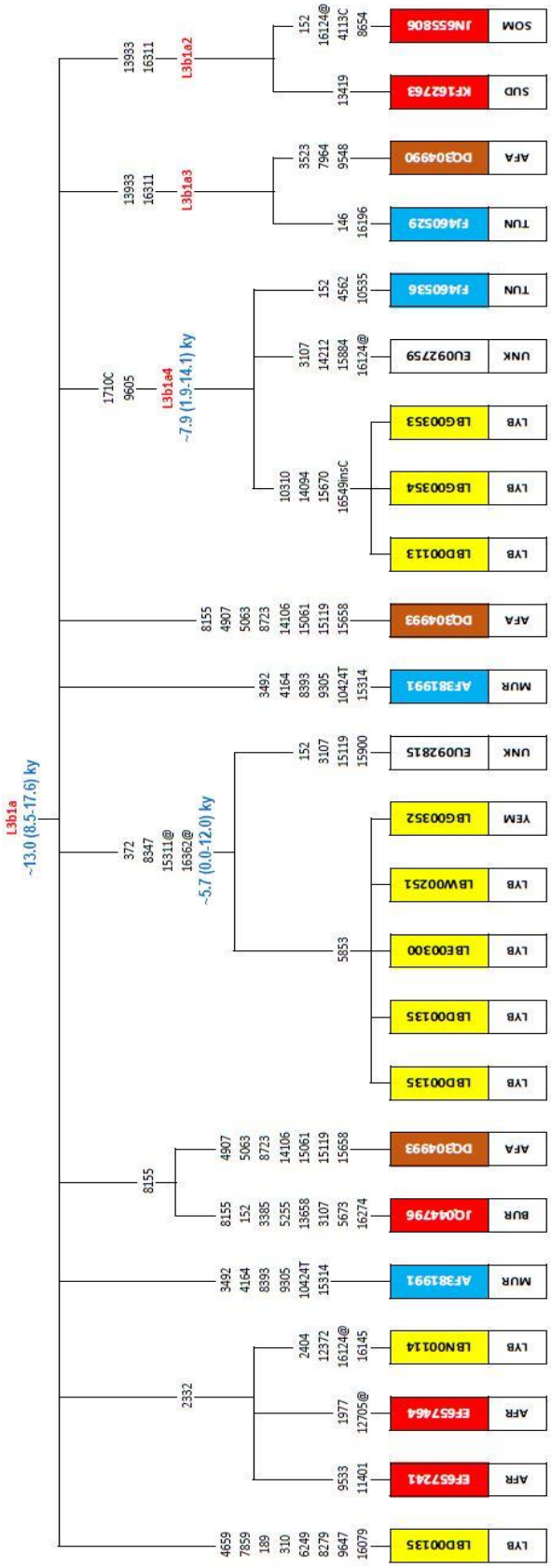

Figure 105. Tree of complete mtDNA sequences of L3b1a lineages

Libya in yellow, North Africa in sky blue, USA in brown, Africa in Red, Europe in blue (LYB, Libya; MUR, Mauritania; TUN; Tunisia; BED; SOM, Somalia; BUR, Burkina; SAF, South Africa; AFA, African American; SOM, Somalia; SUD, Sudan; UNK, Unknown)

A founder analysis was performed for L3 lineages assuming sub-Saharan Africa as source and Libya as a sink of these lineages (Figure 106). The results show a peak at 6.2 kya with *f1* and 4.6 *f2*, that may refer to lineages introduced by trans-Saharan slave trade (Harich et al. 2010). Another peak towards the present may correspond to the recent trans-Saharan slave migration in early nineteenth centuries.

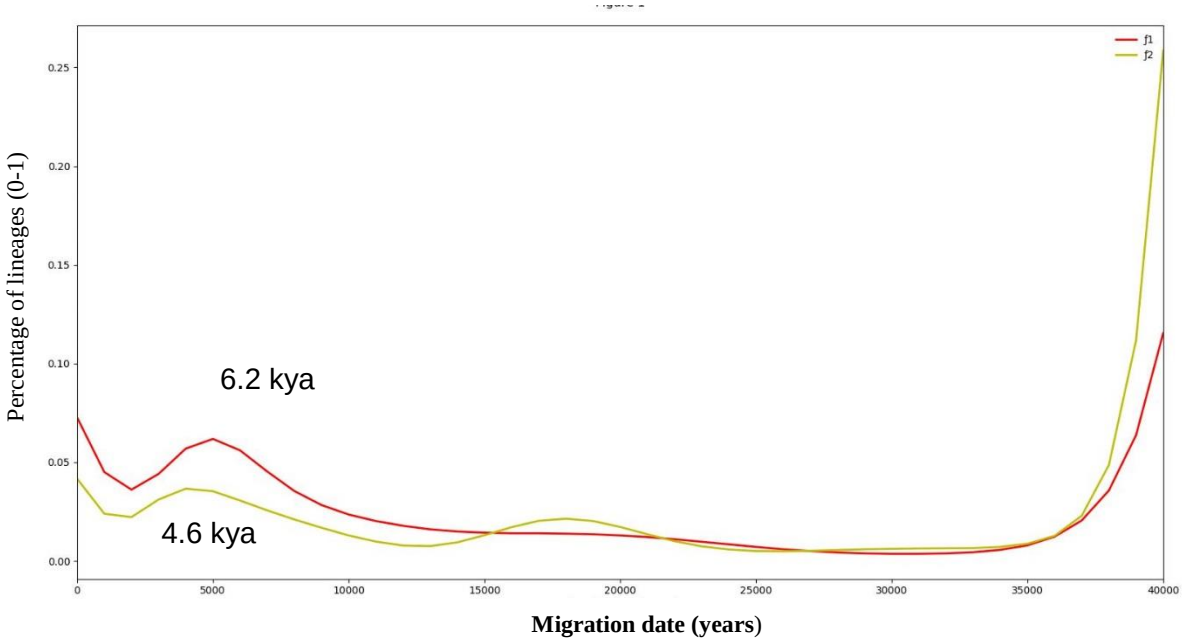

Figure 106. Founder distribution L3 lineages across migration time

Most of the mtDNA haplogroups L found in Libya exhibit a slight east-west gradient distribution. The L1b, L3b, and L3f1b lineages, which have a western African distribution (Harich et al., 2010, Salas et al., 2002) are more frequent in Western Libya suggesting that the origin of most of the sub-Saharan sequences found in Libya might be as a result of the trans-Saharan slave trade routes that were established during recent times in the 7th century (Figure 107). The most likely explanation for the differences in distribution of L haplogroups among our African Libyan results and Libyan Tuareg populations, where the 18% of the L sequences were L0a1, a typical eastern African haplogroup (Ottoni et al., 2009) is a gene flow across the trade routes between North Africa and sub- Saharan Africa, or maybe across North Africa itself. Moreover, differentiation of

mitochondrial DNA lineages in the Libyan Tuaregs could also be due to drift in the Tuareg population. In addition, the presence of L0a lineage in African Libyan is probably a result of the effect of trans-Saharan slave trade routes during the recent time (Figure 107). East Libya is directly connected to western Africa with the Chad Basin, and West Libya is also interconnected with Tunisia, and Algeria, which was, in turn, is connected to other West Africa locations (Figure107), and this may explain how L0a lineage reach Libyan Arabs in East and West. The presence of L1c in African Libyans is probably a result of the effect of trans-Saharan slave trade routes between South Libya and Central Africa.

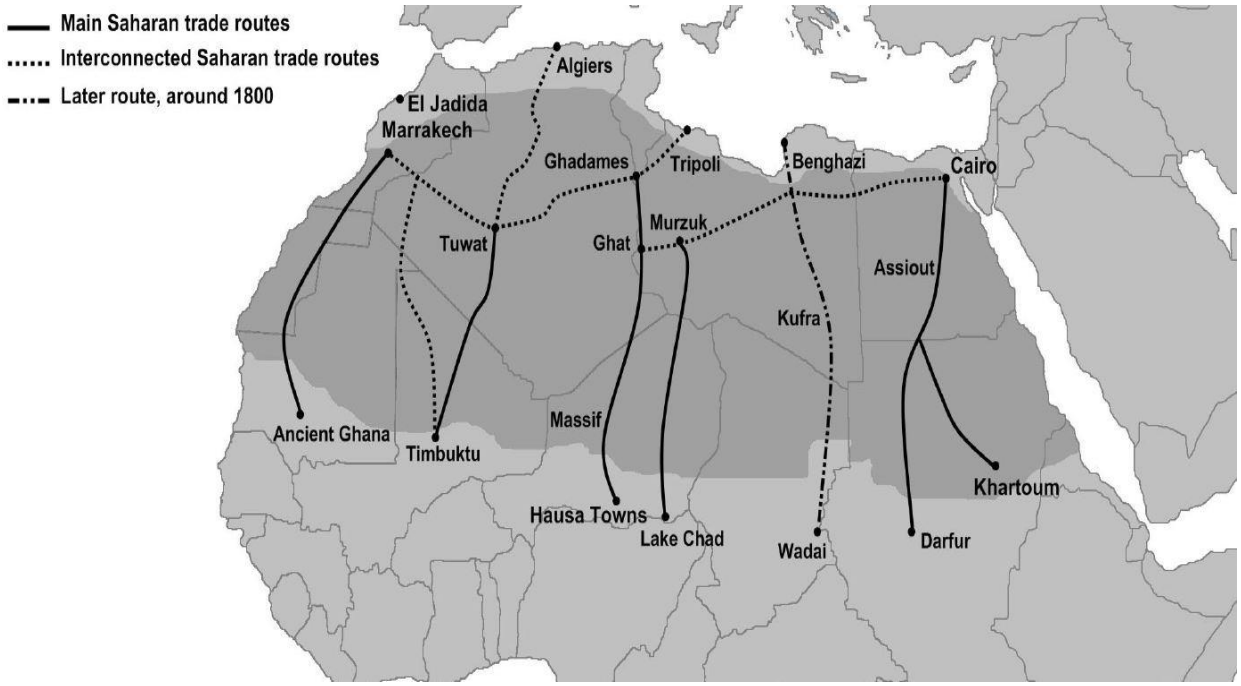

Figure 107. Routes for the trans-Saharan slave trade

Taken from (Harich et al., 2010)

The coalescence ages observed in this study for sub-Saharan lineages shows the young ancestry of these clades, probably arose in sub-Saharan Africa during the Holocene. So the migration of this lineage did not start until the Asiatic horse reached North Africa around 2 kya or even not before the arrival of the first camel-riding Arabs in North Africa, in the seventh century.

4.4. Founder analysis results and discussion.

Founder analysis was done to provide a picture of the genetic contribution of immigration events to the current Libyan mtDNA pool throughout time. It was also performed to answer these questions:

(i) What are the modern-day lineages associated with the first settlements in Libya and are there any sign of sub-Saharan lineages that associated with the Aterian industry among the modern populations?

(ii) Are there signs of contact between Libya and Iberia or south Europe after postglacial climate change?.

(iii) Were there any significant dispersals to Libya in the early Holocene or associated with the farmer expansion in Libya?.

(iv) Are the founder analysis results consistent with the historical events recorded in Libya, especially the major migration, when the Arabs migrated from Arabian peninsula starting from the 7^{th} century?

To answer these questions about Libyan population founders, founder analysis was performed by Dr P. Soares using Bayesian migration partition (BMP) software developed by Dr Soares group at the University of Minho Portugal. The software divides the founder lineages into migration periods (Richards et al. 2000). Libyan were defined as sink populations and the other populations used in this analysis defined as a source population. Also in this analysis, a four migration model was proposed according to *f1* and *f2* results. The first migration wave was found at ~20 kya matching the bump in the scan corresponding to the Iberomaurusian culture. The second wave was at ~11 kya for the beginning of the Holocene to check if there is a sign of Post-Glacial clades. Another historical event occurred at ~6 kya accounts for possible Neolithic arrival. Finally, the most recent event occurred at ~1.3 kya assigned to the beginning of the arrival of Arabs in the 7th and 11th centuries.

The result of the analysis (Figure 108, 109) shows the very similar results for both *f1* and *f2* in that the lineages percentage started to rise ~22 kya in Palaeolithic time accompanied by the Iberomaurusian culture to reach the peak at ~18 kya. This result in agreement with the results obtained by Bayesian skyline plot in this study (Figure 111) and the results seen in the other North African populations. The age estimation and founder analysis results in this study indicated that the lineages associated this expansion were mainly Back to Africa lineages U6 and L2 and HV haplogroups. The curve starts declining until ~ 11 kya then rose slightly in the early of Holocene.This rise in the curve is most likely referred to the lineages expansion from the Iberian Peninsula as a result of the warm conditions post-last glacial maximum that is associated with H1, H3, U5b and V lineages. The remained steady especially for f2, which may be combined with Capsian industry 10‑4.7 kya (Desanges, 1990), the lineages in this expansion probably include the Levant refugee's lineages HV and R0a. The curve then rose sharply starting at 6 kya, most likely associated with the rise of Neolithic and farmer expansion from the Levant and the Middle East, this migration was probably powered by haplogroup R0a, J1d, K and another wave of the haplogroup U6. This expansion in North Africa was also mentioned by (Capelli et al., 2006; Ennafaa et al., 2011; Fadhlaoui-Zid et al., 2011; Frigi et al., 2017). The curve continuously rises to show a major peak toward the present started at 1.3 kya associated with the recent Arabs arriving bringing the rest of Arabian and to North Africa in the 7th century in addition to the sub-Saharan lineages that arrived in recent times.

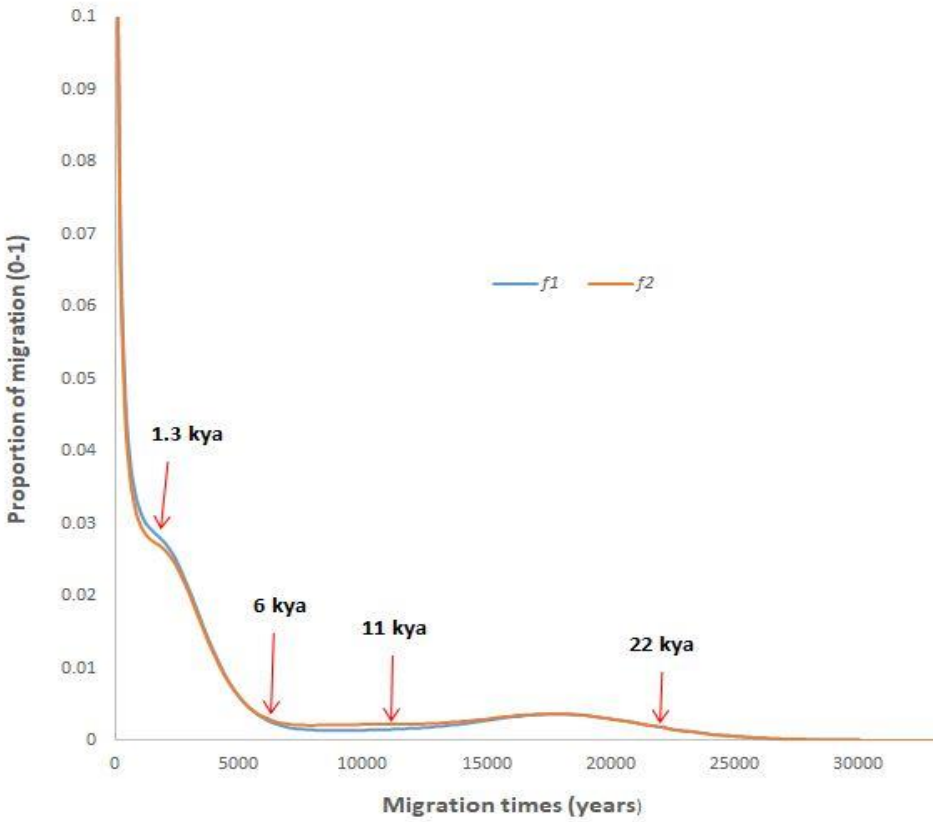

Figure 108. Founder analysis for of Libyan complete mtDNA lineages
Distribution of founder lineages through migration times, time scanned every 200 years. *f1* (blue) and *f2* (orange).

As can be seen in (Table 16, Figures 109, 110), most of the Libyan lineages were concentrated near the present day assigned to the Arabs migration that started ~1.3 kya 68.6% and 65.5% for *f1* and *f1* respectively. The lineages introduced with major Arab movement to North Africa in the 7th and 10th centuries convert the existed people to Islam and changed forever the language situation in North Africa (Najem, 2004). This result is in agreement with picture seen in Libya nowadays where the vast majority of Libyan populations speak the Arabic language and called themselves Arab.
The Iberomaurusian lineages include U6, HV and L2 haplogroups and Neolithic lineages K1a, R0a and J1d participate equally to the Libyan gene pool, 11.7 % *f1* and 11.9% *f2* for the Iberomaurusian lineages and 11.8% *f1* and 12.7% *f2* for the Neolithic transition lineages. Finally, Franco Cantabrian or Levantine refuges only participated by 7.8 *f1* and 9.8 *f2* (Table 16, Figures 109, 110).

The lineages include Iberomaurusian, Franco Cantabrian and Levantine refugees are probably the founder lineages for the Berber populations found in Libya nowadays including Tuareg.

Table 16. The fraction of the Libyan lineages, associated with each of four migrations using (f1 and f2).

Migration time (kya)	*f1*	SE	*f2*	SE
1.3	0.6845	0.0546	0.6557	0.0544
6	0.1189	0.056	0.1271	0.0577
11	0.0788	0.0433	0.0982	0.0457
20	0.1178	0.0351	0.119	0.0356

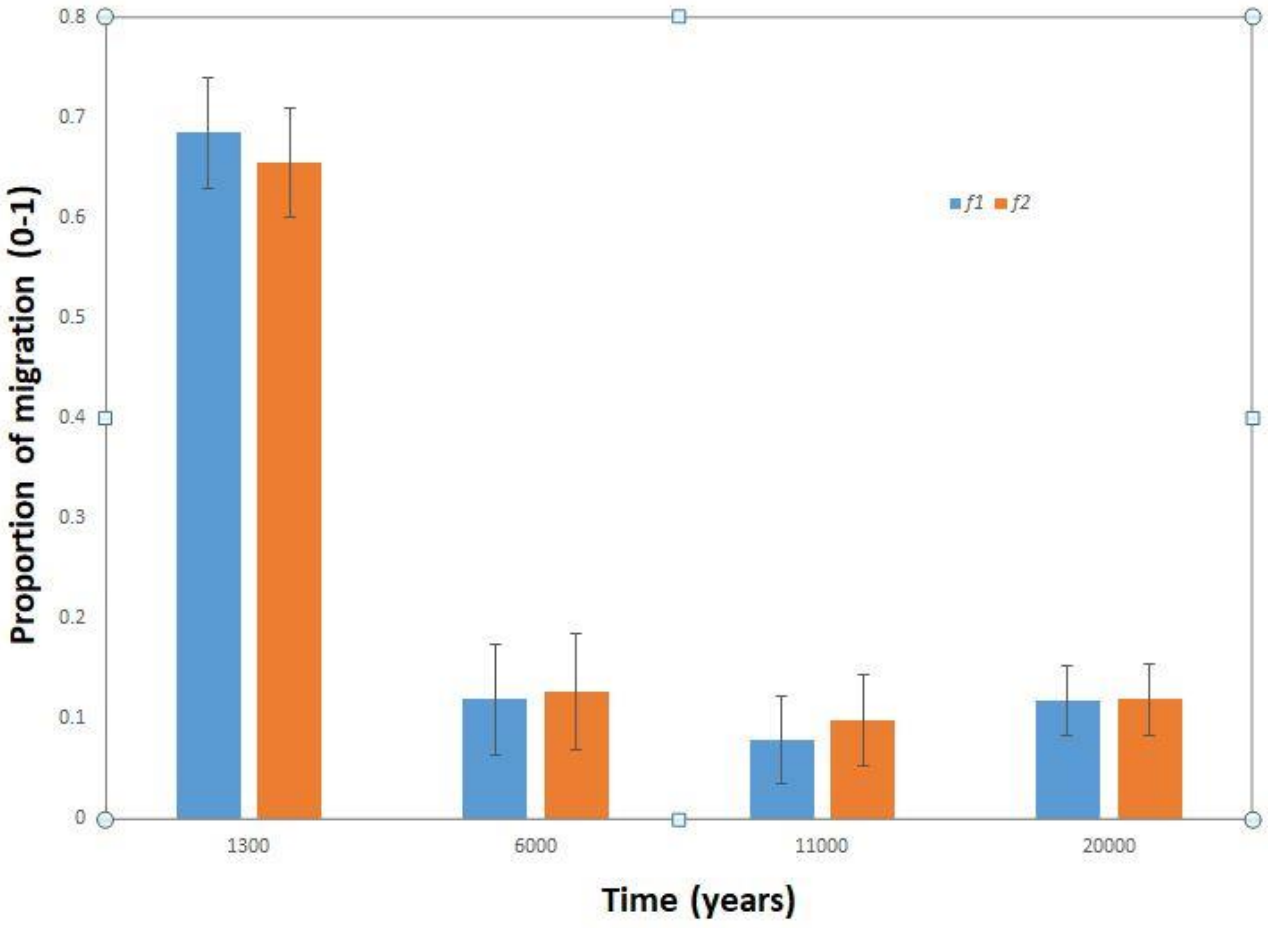

Figure 109. Percentage of the Libyan lineages, associated with the four putative migrations using (f1 and f2).

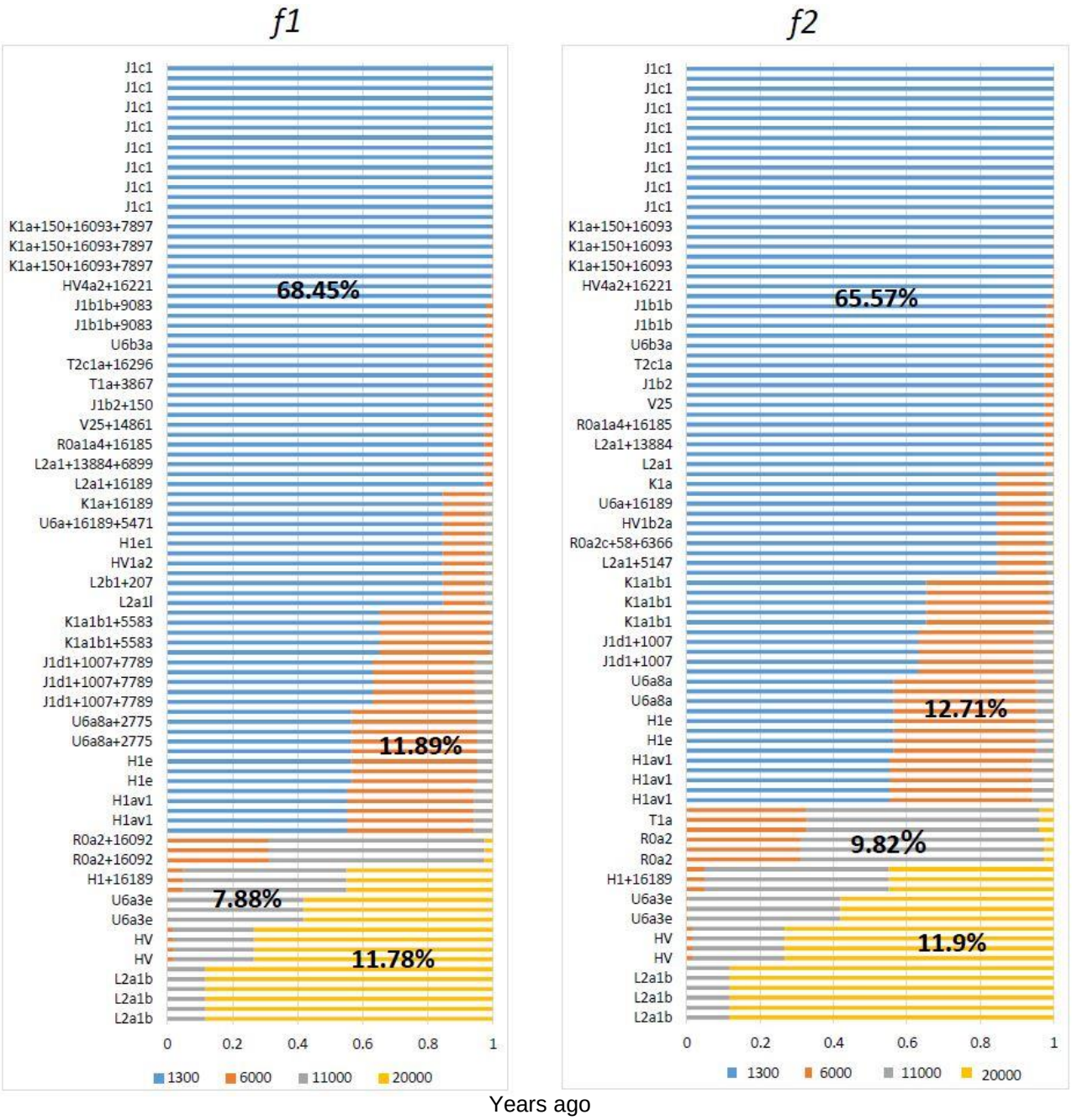

Figure 110. Percentage of the Libyan lineages and complete percentage of each of the four migrations using f1 and f2

4.5. Bayesian Skyline Plot (BSP) results and discussion.

Past population dynamics over time can be inferred from current genetic diversity among modern human using coalescent theory. BSP uses the demographic information in genealogies to build graphical displays. In this coalescent approach, the probability of any two lineages coalescing during a generation is inversely proportional to population size at that time. BSP method does not use pairwise sequence differences like other methods; it is instead applied directly to a group of sequences; therefore there is no loss of information that usually associated with distance-based methods. In an attempt to get more information about past population size changes and the time of population expansion in Libya, Bayesian Skyline Plots (BSP) using Markov chain Monte Carlo (MCMC) sampling algorithm **(**Metropolis *et al***.** 1953**;** Hastings 1970**)** were built with the Beast software (Drummond *et al***.** 2005) for Libyan populations based on entire mtDNA diversity within Eurasian haplogroups that represent 70 % of the total mtDNA gene pool of the Libyan populations. Recent BSP studies in North Africa using haplogroup U6 show that population size was increased moderately until the LGM around 20 kya when the size of the population remains constant for about a 10 kya until early Neolithic before the second growth began and continued until today (Secher et al., 2014). Another study was done by Pereira et al. (2014) using BSP shows that the initial expansion for the back to African lineages started ~22 kya, followed by gradual expansion until ~10 kya. The analysis also showed further growth in the Neolithic time ~5 kya (Pereira et al. 2010). The plot of effective population size performed on Libyan population in this study (Figure 111) represents all Eurasian lineages in Libya. As it can be seen in the plot of population size, the demographic pattern is similar to the demographic pattern seen in the previous studies done in North Africa (Pereira et al. 2010) and agrees with the proposed paleo-climatic fluctuations for North Africa where the population expansion occurs first in the last glacial period. This expansion occurred during the spread of Iberomaurusian culture in North Africa (Figure 111). This expansion likely coincides with the arrival of the back to Africa haplogroups U6 and M1 in Palaeolithic times associated with the Iberomaurusian culture at ~22 kya (Olivieri et al., 2006; Pennarun et al., 2012;

Frigi et al., 2017). The population growth then continued moderately for about 10 kya due to continuous arrival of these lineages and may also be due to the Near Eastern clades J1c, J2a1, T1a1, and T2a1b that arrived by a migration wave from Levant refuge between 16 kya and 12 kya (Pala et al., 2012). This period was followed by the arrival of the Franco-Cantabrian components H1, V and U5b reaching North Africa through Iberian peninsula as result of the warm conditions at the post-last glacial maximum starting at ~10 kya (Achilli et al., 2005; Cherni et al., 2009; Ennafaa et al., 2009; Ottoni et al., 2010; Torroni et al., 2001). It then shows the other significant expansion in the period coinciding with spreading of agriculture during the Neolithic time 5.5 kya when a second significant growth began and extended to the present (Figure 111).

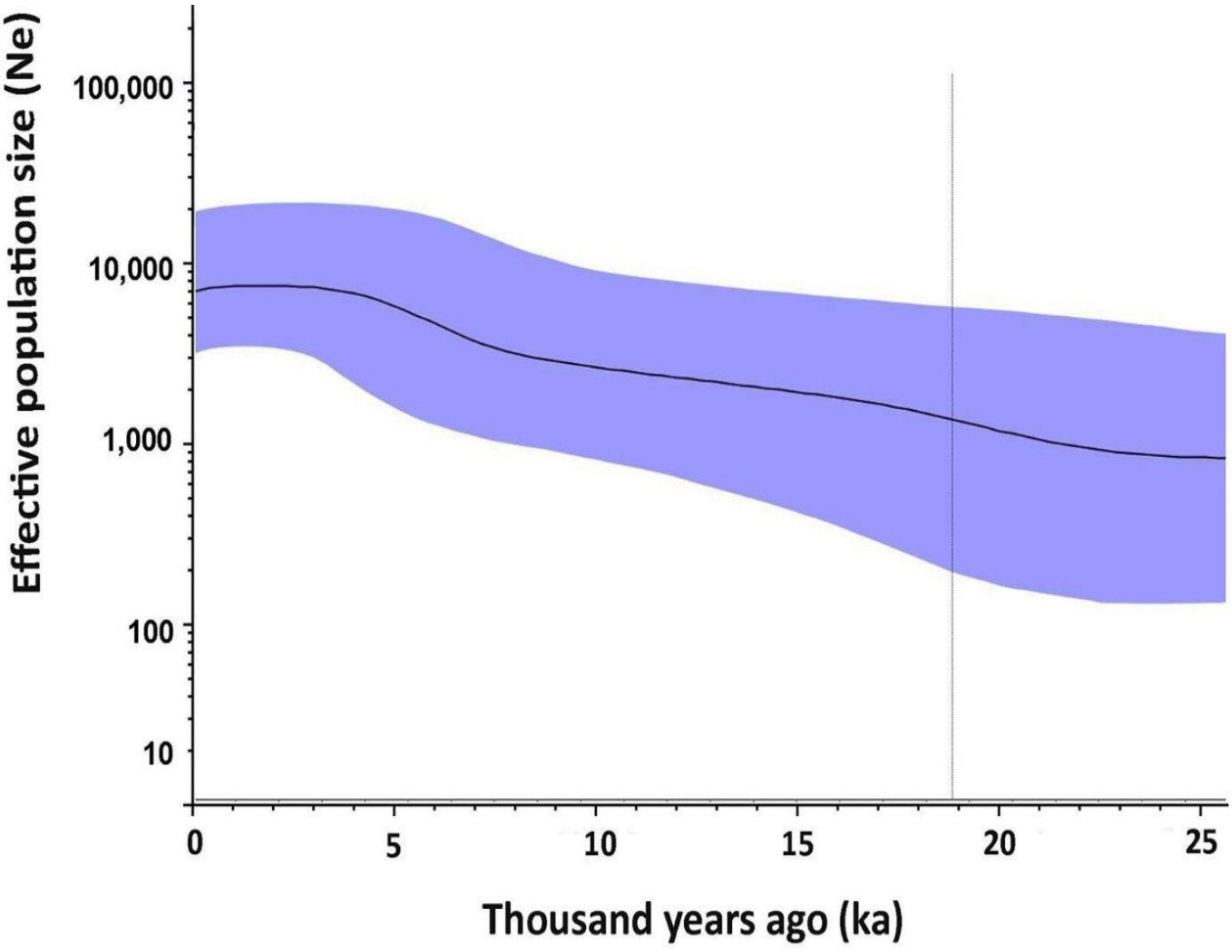

Figure 111. BSPs of effective population size through time inferred from haplogroup N complete sequences in Libya

The black line in the middle refers to the posterior effective population size via time. The blue area represents 95% highest posterior density interval for the effective population size at any time point. 25 years was assumed as generation time.

4.6. Conclusion

The picture for the mtDNA pool seen in the current Libyan population was in accordance with a mosaic structure of the North African population, constituted by Eurasian, Middle Eastern and sub-Saharan components as well as the indigenous North African components which agree with the historical events that occurred in North Africa. This mixture was probably as results to the complex settlement history and cohabitation during centuries between these different populations. The age estimation, founder analysis and Bayesian Skyline Plot (BSP) in this study indicated that the Middle Eastern lineages were introduced in Libya at least since Upper Paleolithic, probably concomitant with the expansion of Iberomaurusian culture starting 22 kya including haplogroups U6, HV, L2 and T1a. Another wave of these lineages has probably arrived during Epipaleolithic and early Neolithic periods associated with the Capsian civilization in North Africa at ~6 kya, this includes J1d and R0a haplogroups. The last expansion of Middle Eastern lineages in Libya occurred in the 7th and 10th century when the Arabs arrived to spread the Islam and Arabic language to the region bringing the other Arabian lineages to Libya. H1, H3, V and U5 haplogroups that are frequent in West-Europe found in Libya supports the post-glacial radiation from the Iberian Peninsula 11kya. The coalescence ages for the sub-Saharan lineages found in Libya nowadays indicated that these lineages did not leave a trace for their settlement ~40 kya and the introduction of these lineages into Libya is in recent historical times, associated with the trans-Saharan slave trade in the seventh century. However, some of the L2 lineages that were especially seen among Ghadames population mostly came to Libya during the Iberomaurusian expansion with these lineages most likely arriving from the Middle East. Also, L1b haplogroup was most likely introduced in Libya and North Africa during the early Holocene after a humid period (Hernandez et al., 2015). In addition, it is obvious from the analysis of the number of shared haplotypes between Libyan groups there is weak genetic flow between different groups even in neighbouring populations such as the Zuwaran population and the Arab populations surrounding them. Geographic isolation, local customs, ethnic

heterogeneity and consanguinity are factors that probably prevented the gene flow between these different ethnic groups (Berber, Arabs and Tuareg and African Libyan) such as.

Chapter 5

Y-chromosome

5. Y-chromosome

The Y-chromosome has extensively investigated all over the world; however, very few studies have been done to investigate the paternal lineages for the current Libyan population. A study on the Tuareg population in south Libya by Ottoni et al (2011) revealed that the Tuareg shared a common North African haplogroup E-M81, haplogroup E1b1b1b predominated followed by a significant percentage of E-U175, haplogroup E1b1a8 (Ottoni et al., 2011). Also, two Y-chromosome studies done on Libyan subjects by Triki-Fendri et al showed that the E-M81 Northwest African haplogroup predominated at (33.7%) followed by Middle Eastern origin haplogroup J(xJ1a, J2)-M304 (27.4%) (Triki-Fendri et al., 2013, Triki- Fendri et al., 2015). Finally, Fadhlaoui-Zid et al., (2013) found similar results to Triki-Fendri et al., (2013) where haplogroups, E1b1b1a-M78 and E1b1b1b-M81, are predominant in North African populations, followed by Middle Eastern haplogroup J. Fadhlaoui-Zid also stated that the most North Africans originated 15 kya during the last glacial warming (Fadhlaoui-Zid et ai., 2013). This part of the thesis aims to identify the genetic composition and distribution of paternally inherited haplogroups in different Libyan populations and groups via 23 Y-STRs loci analysis.

In this study, 219 samples belonging to unrelated males from different ethnic and geographic locations in Libya were analysed 76 were Arab, 132 were Berber (74 from Zuwara, 48 from Ghadames and 10 from Nafusa mountain) and 11 African Libyan. The Promega PowerPlex® Y23 System that allows multiplex amplification and contain four-colour fluorescent detection of 23 STR loci, including DYS393, DYS19, DYS390, DYS391, DYS389I, DYS448, DYS389II, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS439, DYS392, DYS643, DYS576, DYS458, DYS385a/b, DYS456 and Y-GATA-H4. All the results from the samples were assigned to different haplogroups using NEVGEN Y-DNA haplogroup predictor NEVGEN software and confirmed using the phylogeny for the human Y chromosome STRs (Oven et al., 2014).

5.2. Results and discussions

Allele frequencies were estimated by haplotype counting, and the results are showed in the appendix, Table 20. Haplotype diversity was calculated according to Nei's formula while RST using 10000 permutations, was calculated using AMOVA. Y23-STR haplogroups and distribution of Libyan populations are shown in the appendix, Table 19. Percentage of haplotype difference was 90.3% among the Libyan population. The gene diversity among the Libyan population using Y23 STR was found to be high at 0.9998. The power of discrimination capacity (PD) was also calculated for all allele markers. The highest discrimination power (PD) was found in the DYS385b marker followed by DYS458 and DYS390 at 0.934, 0.833 and 0.746 respectively (Appendix, Table 20). The results obtained in this study contributed to the enrichment of the Libyan forensic Y-chromosome databases with a high-resolution PowerPlex 23 Y-STR, especially for familial searching in Libya and estimating the geographical origin of the populations.

5.2.1. Libyan paternal lineages landscape

The haplogroup distribution for the Libyan samples analysed in this study showed a similar picture to earlier studies in Libya (Fadhlaoui-Zid., 2013, Triki-Fendri et al., 2013, Triki- Fendri et al., 2015, Elmrghni et al., 2012) and North Africa (Bekada et al., 2013, Fadhlaoui-Zid et al., 2013, Fadhlaoui- Zid et al., 2011a, Triki-Fendri et al., 2013, Triki- Fendri et al., 2015) (Figure 115). The results showed that the Libyan Y-chromosome haplogroup constituted mainly by indigenous North African lineage haplogroup E- M81 at 44.5%, reaching 53.5% among Libyan Berber followed by Arabian lineage haplogroup J(xJ1a, J2)- M304 at 26% (Table 17, Figures 112, 113, 114). Eastern African clade E-M78 was found at a frequency of 13.5% reaching 26% among Libyan Arabs. Western African clade E-M2 only found at 5% restricted to African origin Libyan. Other less well-represented clades are also shown in Table 17.

Table 17. Y chromosome haplogroup frequency among different Libyan groups

Haplogroup	E-M123	E-M2	E-M78	E-M81	J-M172	J-M267	L-M20	T-M70	R-V88
Libyan	0.0084	0.0504	0.135	0.4453	0.04201	0.2605	0.02521	0.0084	0.0252
Berber	0.014	0	0.07	0.535	0.0422	0.253	0.0281	0.014	0.0422
Arab	0	0	0.268	0.39	0.0487	0.268	0.0243	0	0

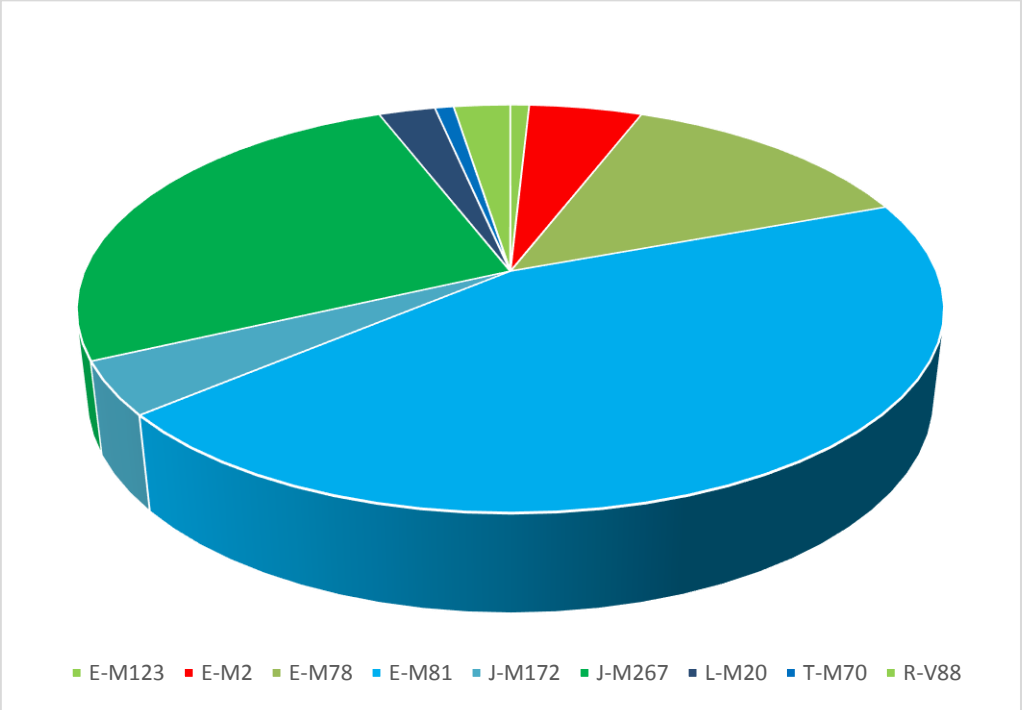

Figure 112. Relative Y- STRs haplogroup frequency among Libyan populations

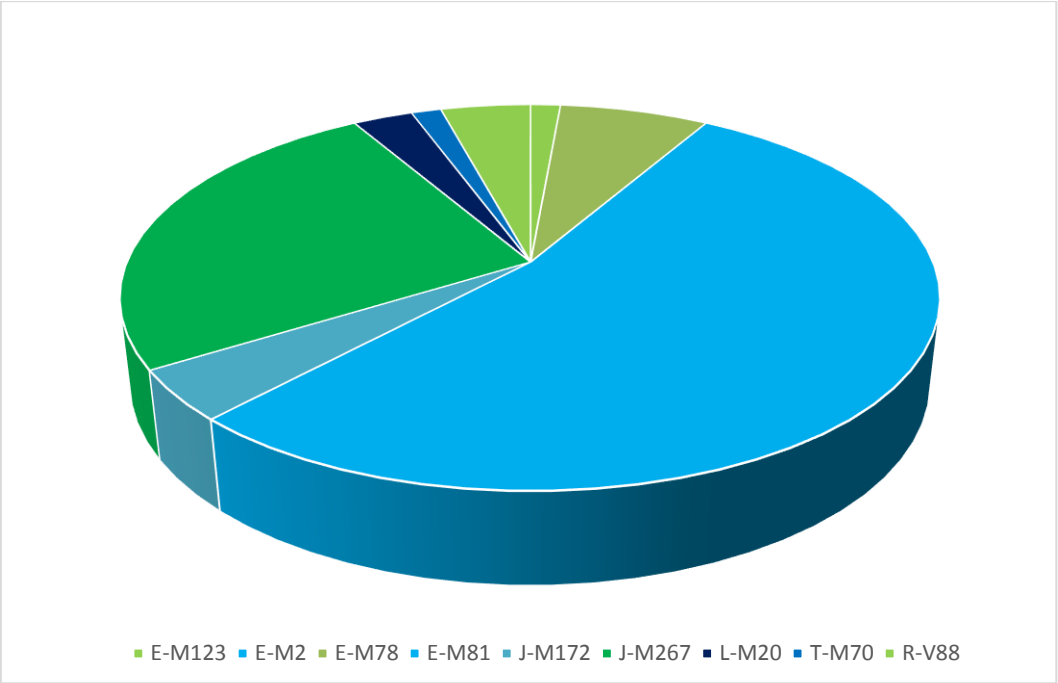

Figure 113. Relative Y- STRs haplogroup frequency among Berber group

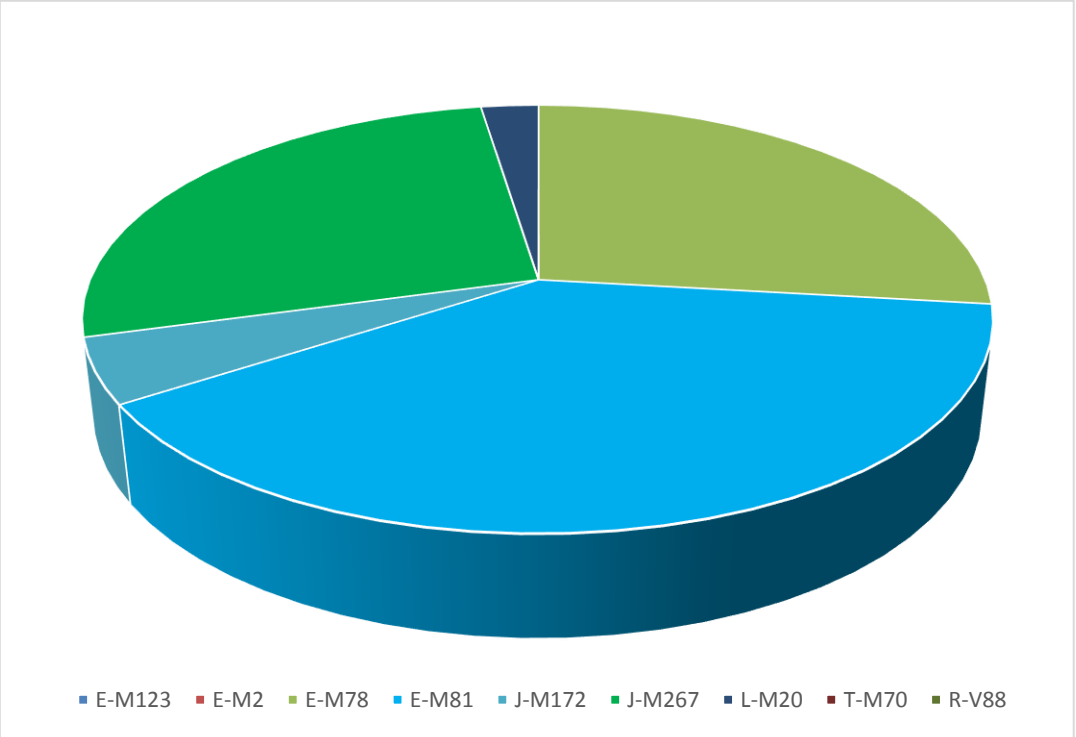

Figure 114. Relative Y- STRs haplogroup frequency among Arabic group

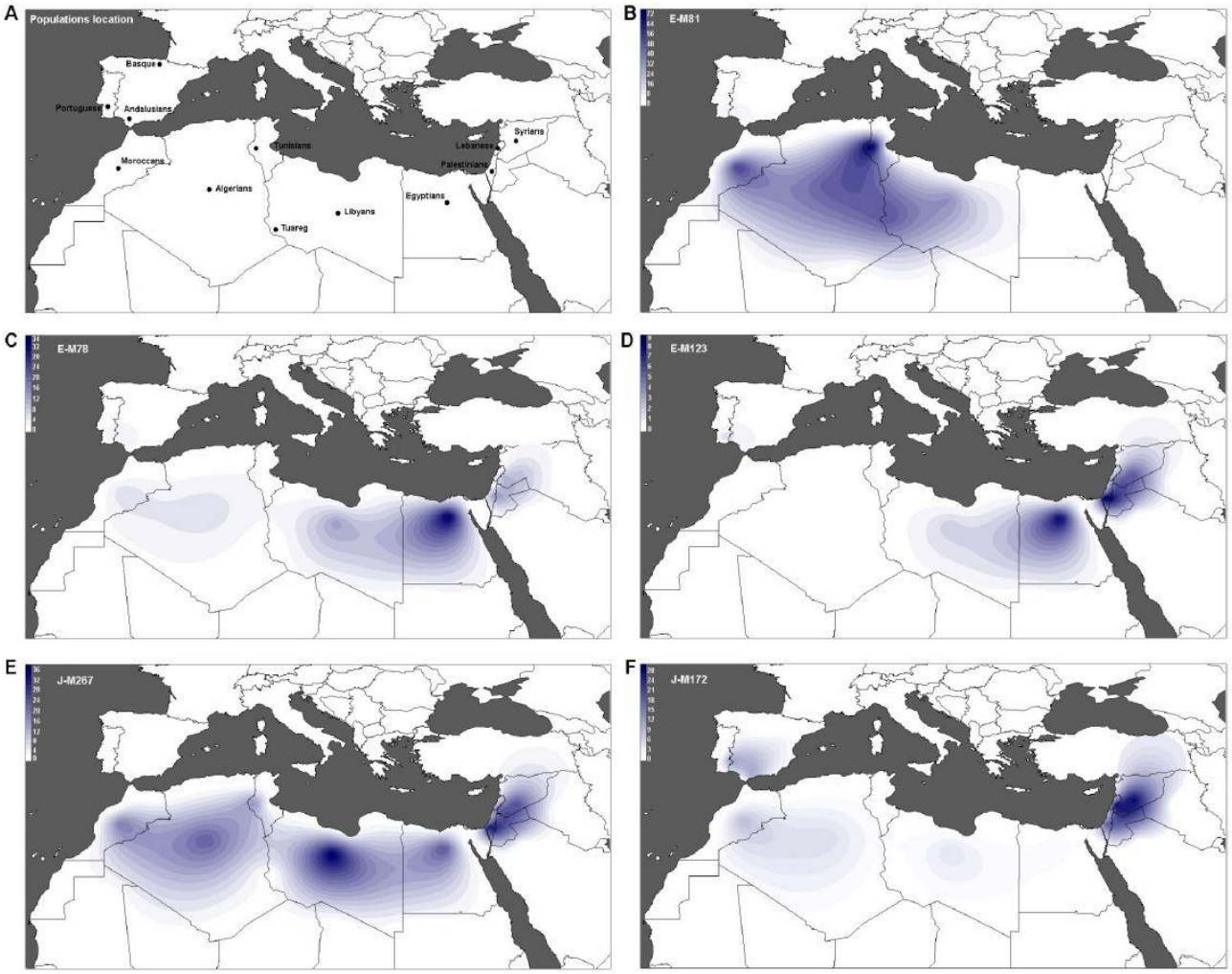

Figure 115. The frequency of the major Y-chromosome haplogroups

Map A shows the location of these lineages; B haplogroup E-M81; C haplogroup E-M78; D haplogroup E-M123; E haplogroup J-M267; F haplogroup J-M172. Generated by (Fadhlaoui-Zid et al., 2013)

5.2.1.1. Haplogroup E

The Libyan Y chromosome gene pool is constituted by North African haplogroups, E1b1b1b-M81 and E1b1b1a-M78 at 44.5% and 13.5% respectively (Table 17, Figures 112, 113, 114), However, they revealed different distribution patterns. While the frequency of E-M78 shows high frequency in East of North African it declines towards West North Africa, E-M81 has been found at high frequencies in west North Africa and decreases towards the East North Africa (Solé-Morata et al., 2017).

E-M81 haplogroup is a typical North African lineage, especially Berber speaking population (Bosch et al., 2001; Cruciani et al., 2002), showed a west-east gradient where it accounts for 76% of males in Morocco and 45.5% in Algeria (Robino et al., 2008) with the North African average at 42% (Arredi et al., 2004). E-81 haplogroup was found in this study among Libyan Berber at frequency of 53.5% and declining to 39% among Libyan Arab (Table 17) and much declined towards East in Egypt and the Levant (Figure 115). It is noteworthy that the distribution of E-M81 chromosomes parallels with the allocation map of Berber speaking populations (Figure 115) (Triki-Fendri et al., 2015). E-M81 is almost rare outside Africa except for Iberia, Sicily and the Middle East. E-M81 reaches the Iberian Peninsula through the Berber Muslims who invaded the Iberian Peninsula in the seventh century (Cruciani et al., 2004; Beleza et al., 2006; Alvarez et al., 2009).

The E-M81 network (Figure 116) show a high level of diversity among different regions where different clades are found in different regions, where the most frequent clades in this haplogroup form a star-like shape in the centre of the network.

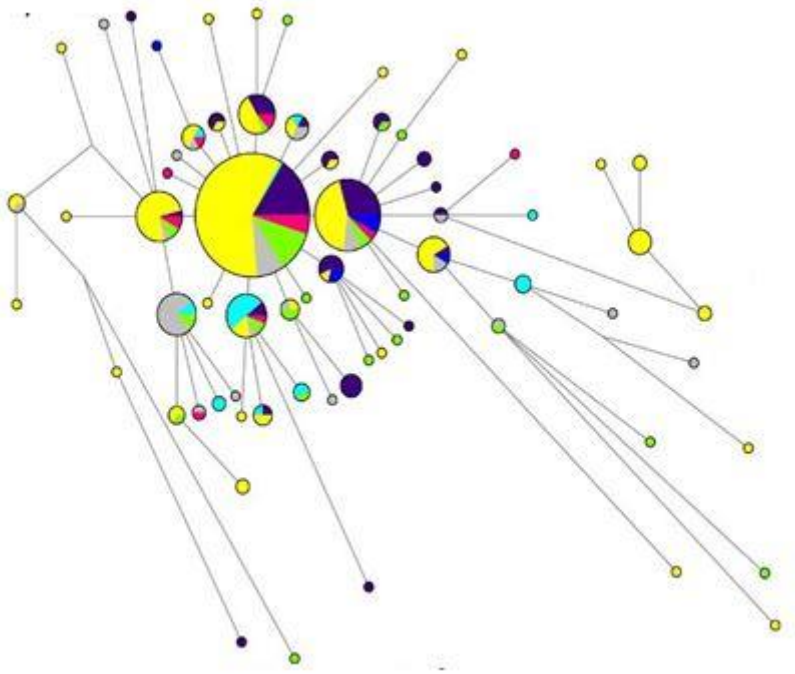

Figure 116. Median-Joining networks of Y-STR haplotypes within haplogroups E-M81
Morocco in light green, Algerian grey, Tunisian in yellow, Libyan Tuareg in light blue, Libyan in purple, Egypt in green, the Levant in blue, Europe in red Taken from (Fadhlaoui et al., 2013)

Among haplogroup E the second most frequent sub-haplogroup found in this study was E-M78 at 13.5% reaching 26% among Libyan Arabs and was only found at 7% in Libyan Berber population (Table 17, Figures112,113,114). E-M78 originated in East Africa (Cruciani et al., 2007) where it was found to be widespread and at high frequency, reaching 77% in Somalia 25.6% among Sudanese and 22.7% among Ethiopians (Triki-Fendri et al., 2015). However, it is also detected in Europe and the Middle East (Semino et al., 2004). Although this clade is only found in the Middle East and North Africa, the network of the E-M78 clade (Figure 117) also show a high diversity.

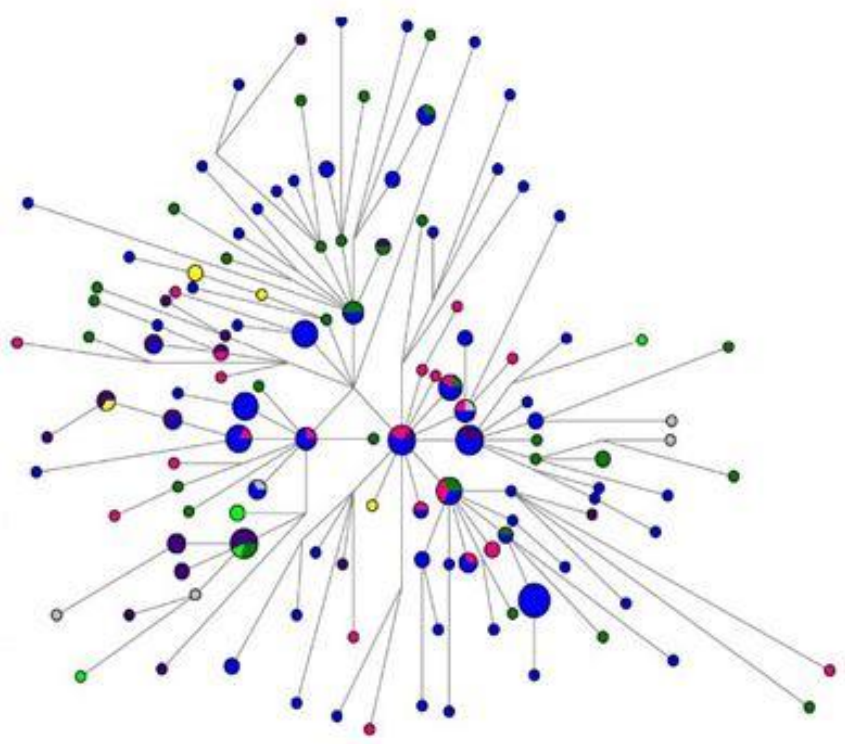

Figure 117. Median-Joining networks of Y-STR haplotypes within haplogroups E-M78

Morocco in light green, Algerian grey, Tunisian in yellow, Libyan Tuareg in light blue, Libyan in purple, Egypt in green, the Levant in blue, Europe in red Taken from (Fadhlaoui et al., 2013)

Also among the E haplogroup, subclade M123 which originated in the Middle East is detected at low frequency 0.84% among the Libyan population and was restricted to the Berber population at 1.4%. This is in contrast to E-M81, E-M78 and M123 which are found at high frequency in the Levant and Egypt and decline towards Northwest Africa (Fadhlaoui-Zid et al., 2013). Although these lineages are predominant among North Africa, the origin and ages of these lineages is still a matter of debate. While some researchers suggested a Palaeolithic age of these lineages (Bosch et al., 2001) others referred to a Neolithic origin (Arredi et al., 2004; Cruciani et al., 2004, 2007, 2010; Semino et al., 2004). However, the most probable age of these lineages is the age suggested by (Fadhlaoui-Zid et al., 2013) where the age of these lineages are much recent.

The last clade among haplogroup E is E-M2 (E1b1a) found in Libyan population at 5% which was restricted to the African Libyan group. The E-M2 lineage is predominantly found in Western Africa and Central Africa and is also detected in North Africa and the Middle East dated to 20-30 kya (Rosa et al., 2007).

5.2.1.2. Haplogroup J

Another abundant component among the Libya population analysed in this study is haplogroup J-M304, which was present at 30.2%. Both two branches of haplogroup J, J-M267 and J-M172 were detected among the Libyan population. J-M267 represented the vast majority of J at 26%, while J-M172 is represented at 4.2% of the Libyan population. Among the Arabic population haplogroup, J was found at 31.7%, with 26.8% for J-M267 and 4.9% for J-M172 haplogroup J was found at a slightly lower per cent at 29.5%, in the Berber population J-M267 at 25.3% and J-M267 at 4.2%. Haplogroup J originated in the Middle East suggesting gene flow from Arabia and the Levant (Semino et al., 2004). It has also been suggested that these lineages arrived in North Africa in association with the spread of agriculture at Neolithic demic diffusion 5.5 kya (Semino et al., 1996).

J1-M267 is found in high frequency at south Arabia where it is found in Yemen at 72.6% with decreasing frequency northwards, Iraq having 56.4% (Al-Zahery et al., 2011) and Egypt 20%, Lebanon 12.5% and Turkey 9% (Semino et al., 2000., Luis et al., 2004., Cadenas et al., 2008). The network of the J-M276 clade from Fadhlaoui et al., 2013 shows that all North Africans also shared the modal haplotype with the Levantines (Figure 118).

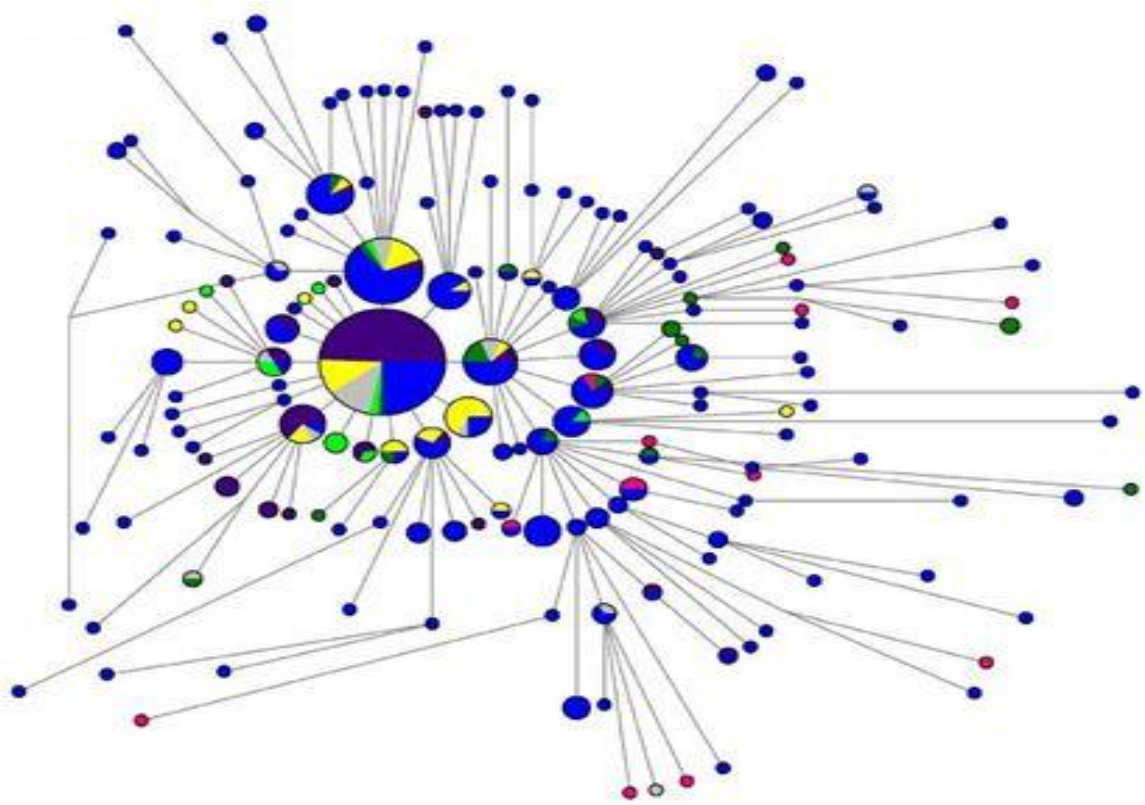

Figure 118. Median-Joining networks of Y-STR haplotypes within haplogroups J-M276

Morocco in light green, Algerian grey, Tunisian in yellow, Libyan Tuareg in light blue, Libyan in purple, Egypt in green, the Levant in blue, Europe in red. Taken from (Fadhlaoui et al., 2013)

The Levantine subhaplogroup J2-M172 lineages display a decreasing percentage gradient toward the Mediterranean and Europe (Fadhlaoui-Zid et al., 2013). It has also has been suggested that this lineage is Arabian introduced to the Levant by Arabs during the Muslim expansion (Semino et al.,

2004; Zalloua et al. 2008). Furthermore, nowadays, this haplogroup shows widespread Mediterranean distribution indicating that this haplogroup is mainly confined to coastal areas (Triki-Fendri et al., 2015). The presence of this lineage among the Libyan population and North Africa, Tunisia (Fadhlaoui-Zid et al., 2011a) confirms the historical records about the migration of Arab Muslim tribes during the 7^{th} and 10^{th} century. This had a major impact on the region and led to Arabization of the region by the imposed culture, of Islam and language (Metz, 1987; Camps, 1996; Cadenas et al., 2008; Zalloua et al., 2008; Tofanelli et al., 2009). Haplogroup J-M172 found at high frequency in Middle Easter at 73.9% and in Europe at 18.5%. It also found in North African populations at 7% (Figure 119). The network of J-M172 network shows that most of the North African samples branched from Middle Eastern clusters (Figure 119).

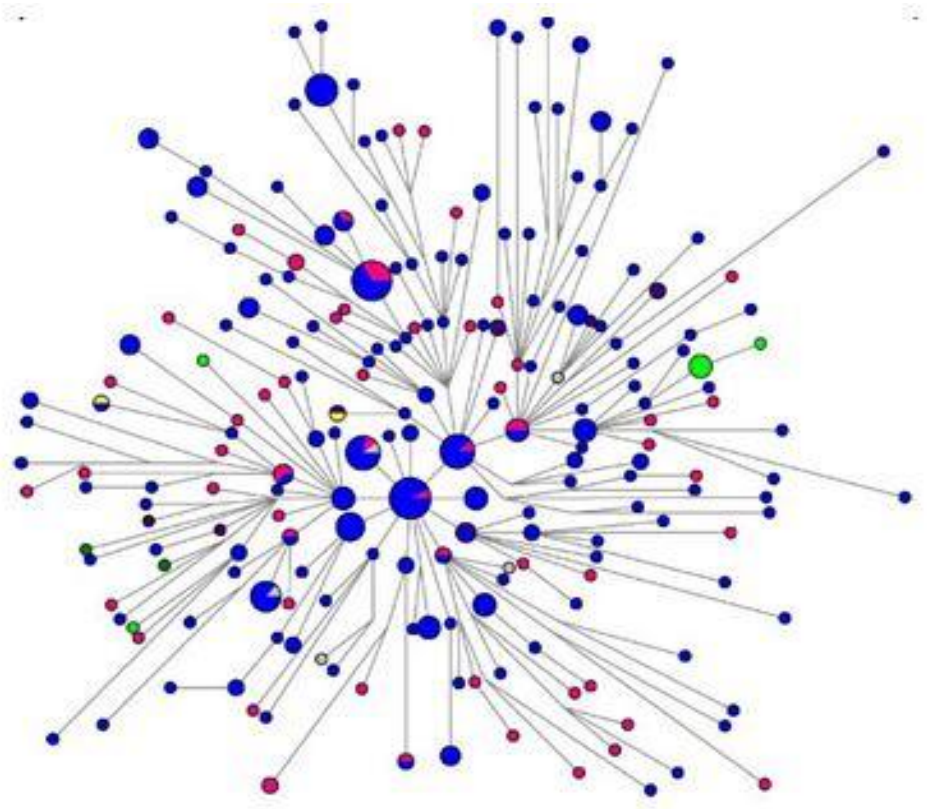

Figure 119. Median-Joining networks of Y-STR haplotypes within haplogroups J-M172 Morocco in light green, Algerian grey, Tunisian in yellow, Libyan Tuareg in light blue, Libyan in purple, Egypt in green, the Levant in blue, Europe in red. Taken from (Fadhlaoui et al., 2013).

In addition to those haplogroups mention above there also minor clades detected among Libyan population in this study including the European haplogroup L-M20 found at 2.5%, Levantine clade R1b V88 at 2.5 and the Mediterranean T-M70 at 0.84%.

Overall, Libyan Y-chromosome gene pool constituted by two main genetic components; The first one is the indigenous populations of North Africa specific haplogroup (Berber print) E-M81 haplogroup that originated from the Berbers, the autochthonous inhabitants of North Africa.

The second, haplogroup J is introduced by Arab Muslims who migrated to the region in seventh-century Table 17.

5.2.2. Y chromosome DNA diversity in Libya in an ethnic and geographical context

In order to visualise the relationships between different Libyan populations based on the Y-STRs chromosome, multidimensional scaling (MDS) plot was performed from the pairwise Rst values. 208 samples used in this analysis in addition to 436 published Y-chromosome STR profiles; 175 from the Western Libyan population (Triki-Fendri et al., 2015), 47 Libyan Tuareg (Ottoni et al., 2011) and 214 individuals from the Eastern Libyan population (Elmrghni et al., 2012). The MDS plot separated the Libyan populations according to their ethnic groups. Although there is a long geographic distance between the two Arabic groups in West and East Libya, they were located in the same place on the chart. Similarly, the Saharan Berber from Ghadames and the Coastal Berber from Zuwara were located close together. The plot put the Tuareg group as expected separated from other Libyan groups. Overall the plot shows that the genetic distances are close between populations within the same ethnic group regardless of their geographic location (Figure 120).

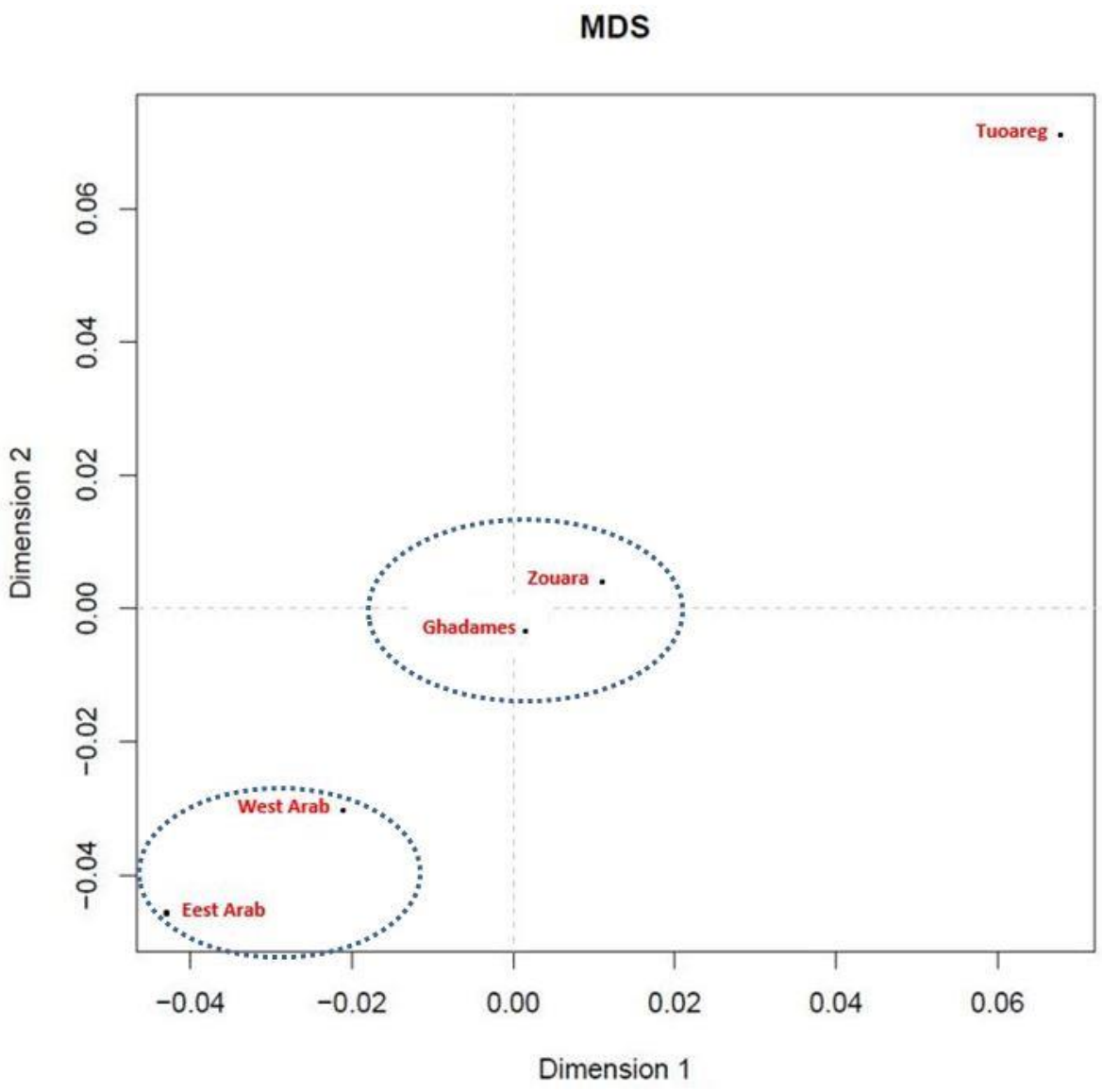

Figure 120. MDS plot based on RST distances between Libyan populations from Y-STR data

To have a general view of the Libyan population's position among other populations, MDS analysis was carried out based on Rst between two Libyan groups (Eastern and Western Libyan) and 10000 STRs profiles from Africa, Arabia, Iberia and Near East populations (Figure 121). MDS clearly separated the sub-Saharan population from the other populations. However, the remaining population clustered according to their geographic location. While the Libyan Tuareg were located in a position separated from other North African populations in the middle position between the sub-Saharan population and North Africa, the western Libyan population showed more affiliation to North Africa whilst the Eastern Libyan show more proximity to Arabia. This result is expected as

the Western Libyan populations also include the Berber population who carry high genetic components belonging to North Africa. However, paternal lineages in Libya show more proximity to the Arabian peninsula than mtDNA (Figures 121,32).

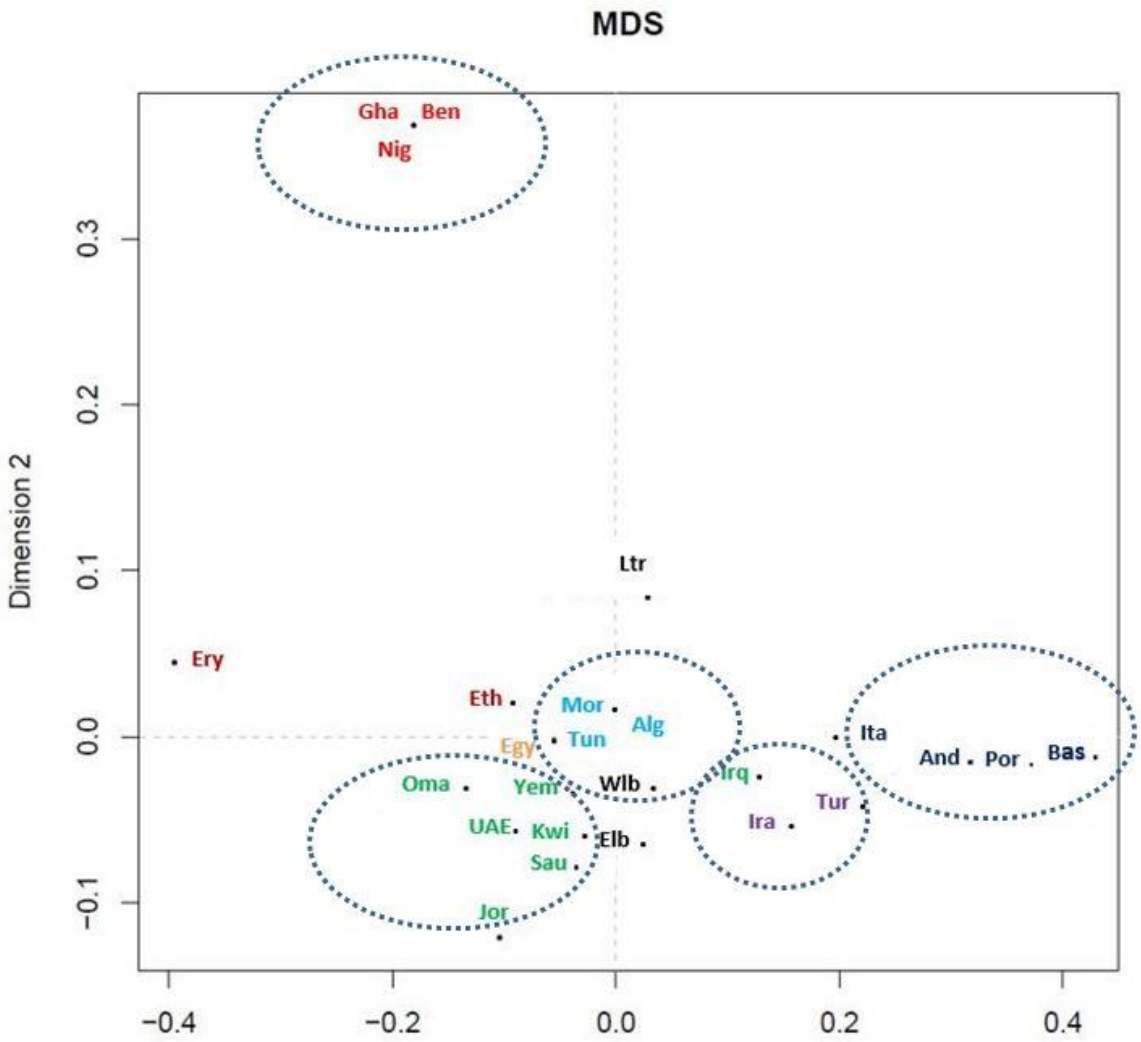

Figure 121. MDS plot based on RST distances between populations from Y-STR data

(WIb, East Libya; EIb, East Libya; MOR, Morocco; Alg, Algeria; Tun, Tunisia; Ira, Iran; ; Kwi, Kuwait; YEM; Irq, Iraq; Sau, Saudia; JOR, Jordan; PAL, Palestine; Gha, Ghana; Ben, Benin; Nig, Nigeria; Sud, Sudan; Eth, Ethiopia; Ery, Eretria; Oma. Oman;Tur, Turkey; Egy, Egypt; Ltr, Tuareg; Ita, Italy; Por, Portugal; And, Andalusia; Bas, Basque)

To have a general view of the position of the Libyan population among worldwide population MDS analysis wae performed based on Rst between two Libyan groups Libyan population and worldwide population, 10000 STRs profiles from Africa, Arabia, Iberian and Near East populations were used (Figure 124). PCA 1 and 2 (axes 1 and 2 in Figure 124) account for 58.53% of the total variation (42.78% and 15.75%, respectively). The population clustered in the chart according to their

geographical location. The first dimension separated the sub-Saharan population from the other populations and were characterised by B-M60, A-M91, E-M2, and E*-M96 haplogroups. The other dimension separated Near Eastern population from the majority of North African. The European population was located on the opposite side of the chart characterised by the haplogroup R (Figure 124). The Libyan population was located in the middle position between North Africa, Arabian and Levant being characterised by North African components E-M81 and E-M78 as well as Arabian J-M267 and E-172 J-M172.

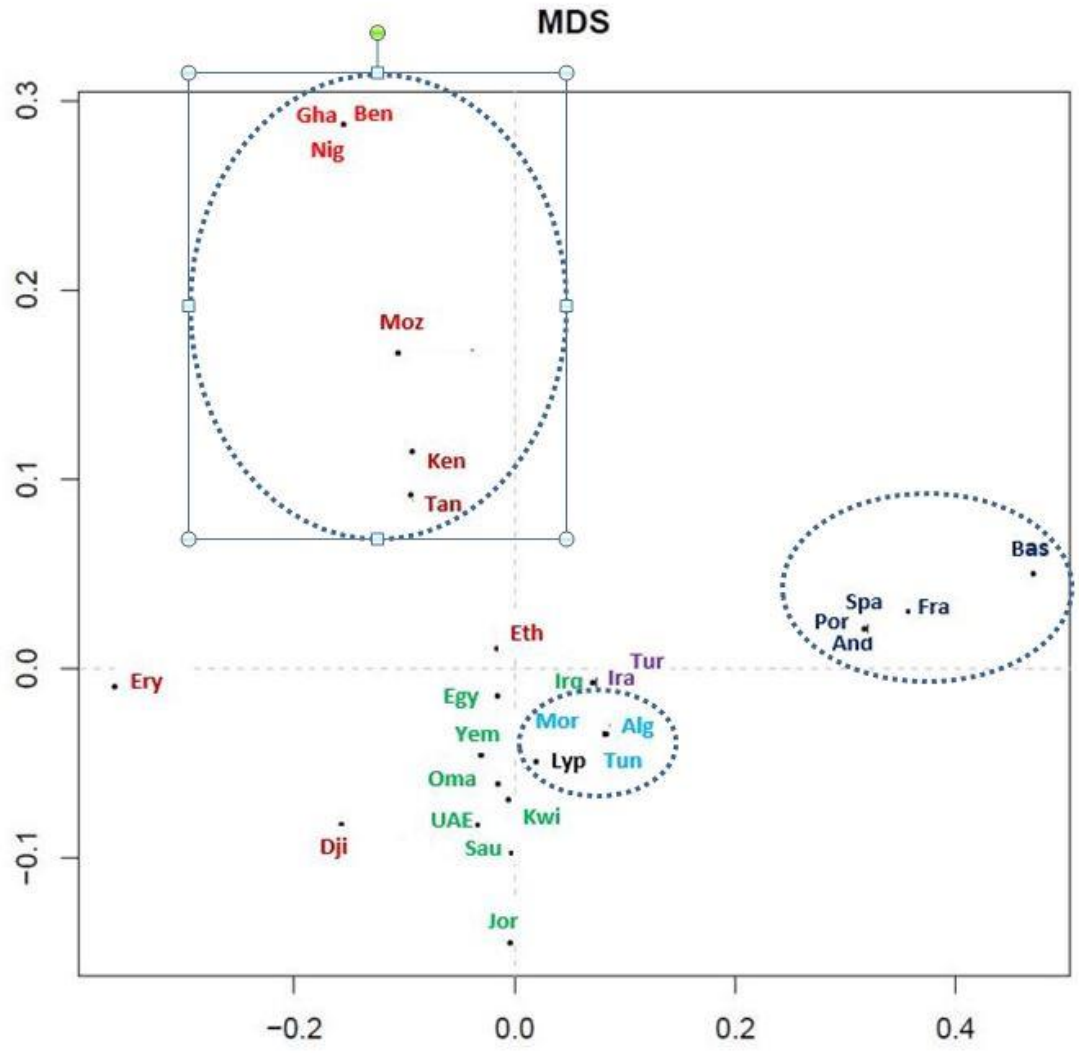

Figure 122. MDS plot based on RST distances between populations from Y-STR data

(Lyp, Libya; MOR, Morocco; Alg, Algeria; Tun, Tunisia; Ira, Iran; ; Kwi, Kuwait; Yem, Yemen; Irq, Iraq; Sau, Saudi; Jor, Jordan; Gha, Ghana; Ben, Benin; Ken, Kenya; Tan, Tanzania; Ken, Kenya; Dji, Djibouti; Nig, Nigeria; Eth, Ethiopia; Oma. Oman; Tur, Turkey; Egy, Egypt; Por, Portugal; And, Andalusia; Bas, Basque; Fra, France)

To have a general view of the position of the Libyan population among worldwide population PC analysis was performed based on haplogroup frequency of population from Africa, Europe, Arabian, Iberian and Near East populations Figure 125. PCA 1 and 2 (axes 1 and 2) accounted for 58.53% of the total variation (42.78% and 15.75%, respectively). The population clustered in the chart according to their geographical location. The first dimension separates the sub-Saharan population from the other population being characterised by B-M60 A-M91, E-M2, and E*-M96 haplogroups. The other dimension separated Near Eastern population from the majority of North African. The European population was located on the opposite side of the chart characterised by haplogroup R Figure 123. The Libyan populations were located in the middle position between North Africa, Arabian and Levant being characterised by North African components E-M81 and E-M78 as well as Arabian J-M267 and E-172 J-M172.

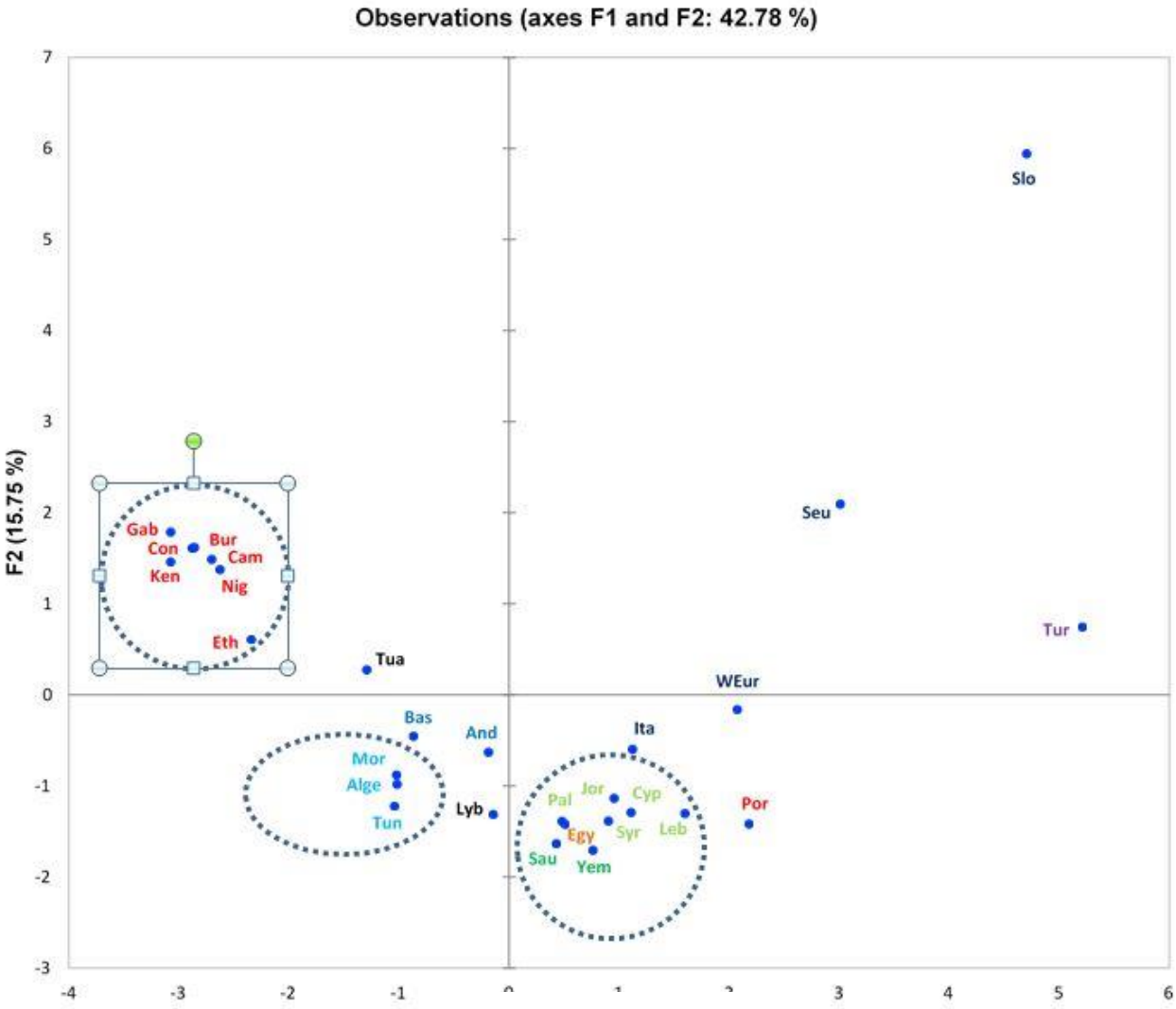

Figure 123. Results of the PCA performed with the Libyan group's data and worldwide based on haplogroup frequencies

(Lyp, Libya; Mor, Morocco; Alg, Algeria; Tun, Tunisia; Ira, Iran; ; Kwi, Kuwait; Yem, Yemen; Irq, Iraq; Sau, Saudi; Jor, Jordan; Gha, Ghana; Ken, Kenya; Dji, Djibouti; Nig, Nigeria; Eth, Ethiopia; Oma. Oman; Tur, Turkey; Egy, Egypt; Por, Portugal; Gab, Gabon; Con, Congo; Bur, Burkina; Cam, Cameron; And, Andalusia; Bas, Basque; Seu, South Europe; Weu, West Europe)

5.3. Conclusion

The results of MDS and PCA analysis on the Y-chromosome STR data obtained in this study revealed homogeneity between Libyans and other North African populations. However, the analysis shows that genetic diversity among Libyan population was higher than other North African populations, where the percentage of haplotype difference was 90.3% and the genetic diversity using Y23 STR was found high at 0.9998.

It can be concluded from this chapter, that genetic diversity among current Libyan populations is a result of waves of human migrations from different directions and at different times. In contrast to mtDNA, Y-chromosome is mainly constituted by two components; The results from this study revealed the dominant Y-chromosome lineages in Libya is a typical of Berber populations, being the indigenous North African haplogroup E-M81 at 44.5% and the Arabian lineage that was probably brought by the Arab people during the 10th century, the haplogroup J(xJ1a, J2) 26%. The Libyan genetic landscape is consistent with North Africa in general and revealed a clear genetic divergence between Libyans and populations from Europe and sub-Saharan Africa, pointing out that the movements of the people were restricted to an East-West direction. This also indicates that the geographical barriers imposed by the Sahara Desert and the Mediterranean Sea were more permeable to maternal lineages than paternal led lineages leading to a different migration pattern between maternal and paternal lineages in Libya. Finally, like, mtDNA lineages the African Libyan and Libyan Tuareg Y-chromosome (Ottoni et al., 2011) show significant differences with other Libyan populations which were explained earlier by genetic drift given due to the isolation of these populations.

Chapter 6

General discussion and conclusion

6. General discussion and conclusion

6.1. Discussion

Due to a scarcity of information about the genetic background of the Libyan population, this study aimed to characterise the mitochondrial DNA and Y chromosome variations among different Libyan populations and various ethnic groups to reconstruct the Libyan history.

In order to achieve these aims, 375 mtDNA D-loop sequence of Libyan from different ethnic groups were characterised and 199 complete mitochondrial DNA sequence of Libyan individuals who belong to the three major Libyan ethnic groups (Arab, Berber, and African) were characterised and analysed. Also, Y chromosome STRs variation of 219 Libyan individuals 17ewere analysed. Intrapopulation diversity structure analyses and the molecular variance (AMOVAs) were also performed. In addition, in order to examine the presence of selection that may affect mitochondrial DNA gene diversity, tests of neutrality (Fu's Fs and Tajima's D) were also performed.

Population phylogenetic analysis was also performed using MDS plots to visualise the relationships between the different Libyan populations. Also, to study the signal of gene flow between the Libyan population and population from North Africa, Arabia, Near East and Europe, MDS plots and PCA were performed. The study also studied the population expansions and their correlation with climate change and technology introduced to the region.

The data obtained in this study were compared with available published data from over 15,000 mtDNA sequences from more than 100 populations, and Y-STRs data of more than 10,000 individuals belonging to more than 70 populations from all over the world.

The data provided in this study will enrich the available knowledge about Libyan population genetics and will ultimately help in the comprehensive understanding of modern human genetic history in North Africa.

Libya's geographic location between North Africa and Near East, in addition to long border with three sub-Saharan countries, Sudan, Chad, Niger make it more subject to more Eastern and sub-Saharan lineages especially East Africa than other parts of North Africa. As expected, the Libyan population, in general, showed higher diversity compared with other North African populations However, Zuwara coastal and Nafusa mountain Berber populations in addition to Libyan Tuaregs have low diversity index similar to other Berber population in North Africa which might be attributable to consanguinity and drift, as these Berber population particular Zuwara and Tuareg live isolated from other Libyan populations. mtDNA markers are more affected by genetic drift, founder events and bottlenecks due to its effective size being 1/4 comparing to autosomal DNA. These effects can be significant when samplings are carried out in small towns such as Zuwara, where inbreeding is very strong.

The variation analysis in this study showed that the variance distribution was higher among groups than among population within groups where a small variation between populations within groups but relatively higher variations among groups. Also, AMOVA analysis showed that profound genetic variations were accounted within each population than among populations which may be explained by gene flow between these populations or as result of sharing the same origin or probably a mixture of the two scenarios.

This pattern of variation also indicates that the genetic variation of some individuals is closer to individuals from another population or groups than individuals from the same population.

From the study, the majority of Libyans who consider themselves Arab are, in fact, a mixture of indigenous Berber and Arab who migrated from the Arabian Peninsula at the end of the 7th century. Only the Berber population escaped this influence such as some tribes that were forced back to the Nafusa Mountains or settled in remote coastal town such as, Zuwara and Ghadames in the south-west Libya border (Najem, 2004, Fadhlaoui- Zid et al., 2011). The majority of Berber who accepted Islam and lost their native language fused with the Arabs in a high degree of melting to the extent of not being able to recognise who is who ((Najem, 2004).

Furthermore, although Ghadames is a relatively small isolated town that contains a small number of the population speaking Berber language; however, their position in main caravan trade road connecting sub-Saharan Africa with North Libya and North Africa with Egypt through Saharan oasis made it subject to gene flow from sub-Saharan Africa and East Africa and Arabia through Egypt. This is also inferred from the mtDNA haplogroup distribution, and age estimation of the lineages among Ghadames population that show an admixture of Berber, Arab and African origin population arrived in town in different periods (Figure 29).

Neutrality test, Fu's FS values which are sensitive to an excess number of alleles were found to have significant negative value among the Libyan populations. This result suggests the presence of a recent population expansion under a bottleneck or a purifying selection. Although the population expansion leads to increase diversity among the population, the bottleneck effect and selective sweeps lead to eliminating the haplotypes from the population and causes a low nucleotide and haplotype diversity, yet the remaining haplotypes are highly divergent (Ramírez-Soriano et al., 2008). One signature of population expansion after a bottleneck is the presence of high haplotype diversity, but low diversity among individuals. As a result, most of the individuals share a one, ancestral haplotype, with several new haplotypes that recently diverged.

However, the Tajima's D values were positive which also indicates population expansions or may reflect the different mutation pattern among the Libyan population, which finally increased the variation among the population. Negative Fu's FS values accompanied by positive Tajima's D (Ramírez-Soriano et al., 2008), may be explained by the presence of a recent population expansion led by the African and Arab groups.

A low level of diversity seen among Zuwara and Nafusa Berber population might be because of low levels of both rare and common mutations as a result of a bottleneck or genetic drift (Ramírez-Soriano et al., 2008). Phylogenetic analysis, PCA and MDS plots of both mtDNA and Y chromosome lineages showed that the populations clustered geographically, and ethnically. The correlation between genetic distances with ethnicity was stronger than with geographic location

among Libya population. Western Arabs in Libya shows proximity with Eastern Arab than geographically adjacent population such as Zuwara and Nafusa Berbers.

Finally, often it is needed to use marker other than the nuclear genome, especially in the case when where nuclear DNA is degraded or in low quantities or poor quality. The high copy number of mt DNA increases the possibility for it to be recovered in a good amount from even a small sample (Butler, 2009). Y-chromosomal data is used for determining the sex of the samples found at the crime scene. Y-STRs are applied to identify the paternal line of the unknown male trace; which is particularly suitable when males and females samples are involved in investigating a sexual assault crime. Y-STRs and mitochondrial DNA are important to complement nuclear DNA. Y-STRs and mitochondrial DNA are important markers cam be used to identify individuals involved in mass disasters and war casualties. Authorities in Libya still struggling to identify thousands of unidentified remains due to civil war in Libya. The mitochondrial DNA diversity and Y 23 STR provided in this study will enrichment of forensic mtDNA and Y- chromosome databases in Libya that are necessary to calculate the match probability of matching profiles.

mtDNA and Y- STR haplogroup profile in Libya.

The phylogeographic analysis of mtDNA and Y chromosome in Libya has shown the presence of a common set of haplogroups shared with the rest of North Africa. Genetic data obtained in this study show that the mitochondrial gene pool ancestry has contributions from four main sources: Indigenous Maghreb paternal lineages E-M81 and E-M78 and maternal lineages U6 and M1 from a (back migration to Africa) that dated to between 22 kya and 12 kya from the Levant. This expansion is supported by age estimation of mtDNA haplogroups U6, M1 and L2 done in this study. Furthermore, the results of both founder analysis through Bayesian migration partition (BMP) software and Bayesian skyline plot (BSP) are also supported this expansion in Libya. The second components are from the European components introduced by hunter-gatherers who moved to North Africa from Franco-Cantabrian refuge through Gibraltar strait after the post-glacial (H1, H3,

V, and U5). In addition to back migration to Africa components, there is another Middle Eastern wave of these lineages has probably arrived during Epipaleolithic and early Neolithic periods associated with the Capsian civilization in North Africa 6 kya, this includes J1d and R0a. These lineages with the paternal lineages E-M81 and E-M78 were most likely associated with the agriculture expansion in Libya and North Africa 6 kya. This is also seen in Bayesian migration partition analysis where the curve of migration started to rais at 6 kya.

The last expansion of Middle Eastern lineages in Libya is powered by Arabian components including J1b, K1a, R0a1a and HV1a occurred in the 7th and10th century when Arabs arrived to spread the Islam and Arabic language to the region bringing the other Arabian lineages to Libya.

The coalescence ages for the sub-Saharan components L0, L1, L2, L3 and L4 found in Libya indicated that the introduction of the majority of current sub-Saharan lineages into Libya was in recent historical times, probably associated with the trans-Saharan trade in the seventh century. However, some of the L2 lineages that were especially seen among Ghadames population mostly come to Libya during the Iberomaurusian expansion in Libya and these lineages most likely arrived from the Middle East. Also, L1b haplogroup is most likely introduced in Libya and North Africa during the early Holocene after a humid period (Hernandez et al., 2015).

The main difference, found through the mtDNA analysis, between the different ethnic and geographical areas studied is the presence of sub-Saharan L lineages in African Libyans compared to other population and southwestern Europeans components in Zuwara population haplogroup such as J1c. African Libyans were characterised by a high percentage of sub-Saharan lineage at 75% (Table 11). The coalescence ages for these lineages among Libyan populations indicated that the introduction of these lineages in Libya probably associated with an Iberomaurusian period such as haplogroup L2 or at least in early Holocene after a humid period for haplogroup L1b.

Taken in to account of the desertification affected the Sahara at least till 5 kya that, made it a strong geographical barrier that prevented the gene flow suggests that the other lineages were introduced to Libya by the Arab trans-Saharan slave trade started in the seventh century and to the major

migration in recent time, 100 years ago. In the other extreme the Zuwara Berber population were characterised by West Eurasian haplogroups components and the J1c1 clade was the most frequent sequence among Zuwara population reaching 32%. Although the Zuwara population may be subject to a genetic drift effect as this Berber population lives isolated among the surrounding Arabic populations. The presence of haplogroups such as H1 and U5 and J1c may indicate contact between Zuwara population and Iberian population after the LGM period.

Moreover, the variations appeared between Libyan groups seemed to be due to the differences in haplogroup distributed among the Libyan groups, whereas haplogroup L is dominant among African Libyans, haplogroup H1 in dominant in Tuareg, U6, U5, T1, J1c is only seen among Berber groups and J1d, U7 J1d and R0a was mainly found in Arabic groups.

It has been also suggested the presence of different patterns of gene flow between females and male in North Africa (Fadhlaoui-Zid et al., 2011b). The difference in maternal and paternal component found in this study is consistent with this suggestion. It seems that both the Saharan and Mediterranean barriers are more permeable to maternal lineages than paternal. Where mtDNA in Libya constituted by important percentage of sub-Saharan and Western European lineages while paternal lineages migration restricted to an East-West direction constituted mostly by Berber-speaking specific haplogroup E-M81 and the Arabic signature haplogroup J-M304.

While the bulk of the slave trade from sub-Sahara was females reaching the maternal genetic pool in Libya, the majority of Arabs who migrated from Africa were a male army which has led to a male-biased Arab effect in Libya.

Both paternal and maternal lineages show strong affiliation of Libyan population to North Africa population; however, Y chromosome showed a more proximity to Arabia and the Levant indicating different levels of the gene flow among two the sexes, as migration was mainly a male contribution.

The characterisation of the Y-chromosome in the Libyan populations shows the presence of both indigenous clades E-M81 and E-M78 and genetic influences from Europe, Arabia and sub-Saharan Africa.

Overall, the results of this study are consistent with the recorded historical events that occurred in Libya and the North African region as a whole. Where the first expansion for the current population in Libya was associated with the spread of Iberomaurusian culture from the Levant during the Palaeolithic time between 22 and 9 kya (Maca-Meyer et al., 2001; Olivieri et al., 2006; Hervella et al., 2016). This expansion was followed by Levant and Franco-Cantabrian refuges components as a result of the warm conditions at post-last glacial maximum and early Holocene starting 11 kya and components. These haplogroups include haplogroups Levant components J2, T1, X1 and Franco-Cantabrian components H1, H3, V, U5. In concordance, analysis of Iberomaurusian bone remains in Taforalt cave in Morocco revealed the presence of haplogroups U6, V, T and probably H (Kéfi et al., 2005). Another expansion detected by the analysis was the presence of Y-chromosome haplogroups E-M81 and E-M78. These lineages diverged around 9.7 kya (Solé-Morata et al., 2011). It also suggested that the majority of North African Y-chromosomal variation is of Neolithic origin (Arredi et al., 2004). The introduction of these lineages probably associated with the Capsian industry 10–4.7 kya (Desanges, 1990) that persisted after the adoption of agriculture 5.5 kya in the North African region.

However, the biggest expansion in Libya occurred by the 7th century when the Arab Muslims arrived in Libya to spread Islam and Arabic language (Fadhlaoui- Zid et al., 2011b, Ennafaa et al., 2009). The arrival of Arabs paved the way to the appearance of south-Saharan L lineages that are attributed to the recent Arabic slave trade, starting in the 7th century CE, characterised by trans-Saharan migrations (Harich et al., 2010).

1.16.2. Berber settlement in Libya

Berbers are the indigenous peoples of Libya and North Africa. Several studies have been done to study the population time settlement of the region based on the distribution of mtDNA of haplogroup U6 as it is the haplogroup associated with the migration of Berber population. Many theories were suggested regarding the settlement of human populations in North Africa. One theory

suggested that the U6 spread to North Africa from Southwest Asia. Other theory supposed that M1 and U6 were introduced to the region 40-45 kya in association with the spread of Dabban culture (Pennarun et al., 2012). It is also proposed that the lineages be introduced to the region through multiple waves of migration between LGM and the Holocene (Pennarun et al., 2012) with a major expansion of the U6 in Megreb at 22 kya associated Iberomaurusian culture (Pereira et al., 2010). The data obtained in this study from the founder analysis and Bayesian skyline plot (BSP), as well as the age estimation of the U6 and M1, do not agree with an earlier settlement of the Berber population during Dabban technology. Instead, it supports multiple migration waves started 22 kya. So the current Berber population are a mixture of several lineages include, Iberomaurusian, hunter-gatherer from Franco Cantabrian refuge and Levant refugee.

1.15.11. **Last Glacial Maximum expansion.**

Archaeological studies suggested that the Franco-Cantabria refuge was possibly the main source of modern Europeans at the Holocene 11.5 kya after the end of the Last Glacial Maximum (LGM), around 13 kya (Cherni et al., 2005, Ennafaa et al., 2009, Fadhlaoui-Zid et al., 2011a). This expansion toward Europe is associated with spreading maternal lineages haplogroups H1, H3, U5b and V (Cherni et al., 2005, Ennafaa et al., 2009, Fadhlaoui-Zid et al., 2011a).

Furthermore, the hunter-gatherer expansion was not restricted toward Europe but also towards the south to the North Africa (Achilli et al., 2005; Cherni et al., 2009; Ennafaa et al., 2009; Rhouda et al., 2009; Ottoni et al., 2010). Although there is no archaeological evidence in Libya support a theory of population flow between Iberian peninsula and North Africa, the presence of these lineages in Libya especially among Berber populations (Figures 29, 30, Tables 11, 18) and ages estimated for these lineages in this study further support this suggestion and that the Europeans especially Iberians left their genetic print by mixing with Berber population at least since the Epipaleolithic time 11 kya. On another hand, the Levant refuge in Near East is also another source of the European hunter

gatherer, where they spread into Europe after the last glaciation, 19 kya with major expansions between 16 kya and 12 kya (Pala et al., 2012). Although there no archaeological evidence supporting this expansion, the presence of subclades of haplogroups J1c and T1 in Zuwara population like T2 in the Ghadames population (Figures 30, 31 Table1s 11, 18) and their ages confirms that this spread occurs in the period, between 16 kya and 12 kya (Pala et al., 2012).

Also, the presence of Y-chromosome E-M81 and E-M78 and mtDNA haplogroup T2 in Libyans (Table 18) indicate another wave of expansion between 11 kya and 7 kya (Pala et al., 2012). Moreover, the distribution of haplogroup-T haplotypes among Tunisian Berber Takrouna, Chenni-Douiret and Jerbian Berbers (Frigi et al., 2017b) as well as haplogroups T1 and J1c among Zuwara Berber in this study and the presence of highest frequencies of T haplogroup in in Egypt , indicates the Levant refuge was the source of these lineages among North African Berber at least since late glacial period.

6.2. Conclusion

This study provides the first data sets of complete mtDNA genomes and Y-chromosome 23 STR loci; in addition to mtDNA D-loop of 375 sequences from Libya. In the current study, 199 whole genome mtDNA variations with diverse mtDNA haplotypes were analysed. Consistent with the previous studies of mitochondrial in North Africa, the results of this study further supported the evidence for recent population expansion in Libya. Also, the analysis of mitochondrial DNA in the current study has shown the dominance of Eurasian lineages in Libya. Moreover, the overall picture for the mtDNA gene pool shows a mosaic structure contains lineages originated in different places and arrived in different periods of time including the Middle Eastern, West European, and sub-Saharan African. In the case of Berber populations this includes the Zuwara Berber and Tuareg, it shows that they have experienced high levels of genetic drift resulting in a small number of haplotypes elevated to high frequencies as demonstrated by haplogroups J1c for Zuwara and H1 in Tuareg. However, paradoxically, given their status as relative newcomers to Libya much higher levels of diversity remain among the Arabic populations. Founder analysis results show that some of the Berber ancestors could have involved in the first colonization in Libya 22 kya during Iberomaurusian period; however, it is apparent that these lineages only represent a small portion of the current maternal lineages in Libya. Berber populations appear to descend from indigenous Iberomaurusian populations and have received multiple maternal lineages from West Europe (Franco Cantabrian lineages), Levant components during the Holocene and in Neolithic associated with the spread of agriculture. Due to the position of Ghadames in trade route in the pre-Sahara connecting Sub-Sahara and North Libya and Siwa oases in Egypt with North Africa, it composed a relatively higher percentage of Arabian and East African components than other Berber populations.

Despite the large cultural impact of the Arab expansion in Libya and North Africa, there is a small influence of these lineages in Libyan mitochondrial gene pool.

The assumption of earlier African Mousterian during Aterian or spread of Dabban culture 40-45 kya was not evident among the current Libyan population. Instead, the majority of sub-Saharan lineages that was found among African Libyan support the genetic influence of recent trans-Saharan slave trade initiated by the Arab Muslims in the 7th century. However, the African lineages found in Berber populations such as L1b and L2a1 were introduced into Libya during the early Holocene and associated with Iberomaurusian respectively.

The current study was also able to characterize haplotype diversity for Y-chromosome 23 STR loci for 215 Libyan individuals. The analysis showed that the Libyan population are correlated with their geographical region, North Africa including Egypt. The male genetic pool in Libya mainly constituted by dual components; indigenous North African lineage, typical Berber population E-M81 (54%), and the Arabic signature haplogroup J(xJ1a, J2) (26 %) that likely spread by the Arab Muslim soldiers in the 7th century.

The clear genetic variance in the paternal lineages between Libyan population and sub-Saharan and European is probably attributed to the Mediterranean sea and Saharan barriers that made the movement restricted an East-West direction

By comparing the results obtained from haplogroups analysis, MDS and PCA plots for both mtDNA and Y-chromosome, it can be concluded that both lineages experience different patterns of gene flow. While maternal lineages in Libya show discontinuity with Egypt and Arabia, the male lineage was consistent with Egypt and other North Africa and show that paternal lineages were influenced by Arabian lineages more than mtDNA.

Furthermore, the analysis shows that the distributions of some lineages are also statistically correlated with ethnicity, for instance, mtDNA haplogroup L and Y-chromosome haplogroup E-M2 among African Libyan, J1b, R0a and U7 among Arab populations and mtDNA haplogroups U5, T1

and U6 among Berber populations. In contrast, the African Libyans were characterised by a high percentage of sub-Saharan lineages.

Overall, the new data presented in this study may serve as a base for comparison with other populations and may contribute to the establishment of databases suitable for human evolution studies and forensic casework.

6.3. Future Work

- The next step in this research is to maximise the number of NGS samples to include all Libyan regions especially southern part of Libya, ethnic backgrounds, and minority groups such as Tube, Tuareg, Turkish and Greek Libyans, paving the way for the construction of mtDNA database for use in human evolution studies, medicine and forensic applications.
- Due to the limited profiles available for Y-23 STR for North Africa, middle east and sub-Saharan population, constructing the global phylogenetic tree using reduced-median networks of 23 STR Y chromosome will be useful to study paternal haplogroup diversity and origin among Libya population.
- As recently, there has been a drop in the costs of generating whole human-genome SNP data; analysis of the variations among Libyan population using this technique will be informative to clarify the demographic history Libyan population as well in the study of the genetic diseases.
- Analysis of the current Libyan population especially the Berbers provided some insights into early human movements in Libya. However, the information available is ineffective in resolving questions about ancient human expansions. Therefore the analysis of ancient indigenous DNA from human fossils is crucial to constructing of modern Libyan human history.

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8. Appendix

Table 18. Mitochondrial DNA sequences of the 375 Libyan individuals analysed in this study

The data show the ID, geographic localisation, and hyplogroups and subclades frequency for each ethnic group analysed (AFL, African origin; EAR, Eastern Libyan Arab; WAL, Western Libyan Arab; NAS, Nafusa Berber; Zou, Zuwara Berber; Gha, Ghadames Berber)

No		Hg		Hg	Possible Subclade
129	AFL	CR	114 129 148 168 172 184 187 188 189 223 230 311 320 519 93 152 185 189 194 195 236 24 263 315.1	L	L0a1
130	AFL	CR	148 168 172 187 188 189 223 230 311 320 64 519 64 93 152 185 189 236 247 263 315.1	L	L0a1a
133	AFL	CR	086 126 187 189 223 245 247 264 270 278 293 311 519 73 152 185 189 195 247 263 315.1	L	L1b1a
134	AFL	CR	038 129 187 189 223 278 293 294 311 360 519 73 151 152 182 185 186 189 195 198 247 263 297 315.1 357	L	L1c1d
138	AFL	CR	124 223 278 311 362 519 73 263 315.1	L	L3b1
137	AFL	CR	145 223 278 362 519 73 263 315.1	L	L3b1a8
147	AFL	CR	093 223 265 519 73 150 195 236 263 315.1	L	L3e3
148	AFL	CR	092 214 223 265 519 73 150 195 263 315.1	L	L3e3
150	AFL	CR	188 189 223 274 292 293 311 316 335 362 399 519 73 146 152 195 244 263 315.1	L	L4b2b1
125	AFL	CR	145 176 223 362 390 519 73 152 204 263 315.1	N	N1b1
121	AFL	CR	294 304 263 315.1	R0	H5a4
119	AFL	CR	519 263 315.1	R1	H
120	AFL	CR	179 189 194 195 197 519 73 189 195 263 315.1	R2	H
122	AFL	CR	172 219 278 73 152 263 315.1	R3	H5a4
288	EAR	CR	189 519 263 264 315.1	R4	H
283	EAR	CR	189270 465 270 519 73 93 150 195 263 309.1 315.1	R5	H1
287	EAR	CR	111 93 263 309.1 309.2 315.1	R6	H49a2
289	EAR	CR	093 300 362 482 73 239 263 309.1 315.1	R7	H6b
272	EAR	CR	069 128 193 263 300 309 73 152 263 295	J	J1d1a
273	EAR	CR	069 126 193 300 309 73 152 215 263 295 309.1 315.1	J	J1d1a
276	EAR	CR	069 126 519 73 150 152 189 195 263 295 315.1	J	J2a
277	EAR	CR	069 126 231 319 73 150195 263 295 315.1	J	J2a2c
278	EAR	CR	069 093 126 231 319 73 150 195 263 295 309.1 315.1	J	J2a2c

292	EAR	CR	093 224 73 150 189 263 309.1 315.1	K	K1
293	EAR	CR	224 311 519 73 146 195 263 309.1 315.1	K	K1b2
298	EAR	CR	189 223 264 278 311 519 73 152 182 185 189 195 247 263 315.1 357	L	L1b1a18
299	EAR	CR	126 187 189 223 264 270 278 292 293 311 519 73 152 185 189 195 247 263 315.1 357	L	L1b1a18
301	EAR	CR	223 278 362 519 73 152 263 315.1	L	L3b1b1
304	EAR	CR	092 214 223 265 519 73 150 195 263 315.1	L	L3e3
305	EAR	CR	209 223 292 311 519 73 152 263 309.1 315.1 365	L	L3f2a
306	EAR	CR	126 187 189 223 264 270 278 311 519 73 152 263 309.1 315.1	L	L3h1a2b
307	EAR	CR	186 189 192 223 256 284 311 73 189 263 315.1	L	L3h1b1
308	EAR	CR	186 189 223 192 256 284 311 73 189 263	L	L3h1b1
296	EAR	CR	145 176 223 311 390 519 73 152 263 315.1	N	N1b1
279	EAR	CR	126 294 296 519 73 263 309.1 315.1	T	T2
327	GHA	CR	519 263 315.1	R0	H1g
325	GHA	CR	223 327 73 150 189 193 200 263	R1	H
324	GHA	CR	067 343 519 263	R2	HV1
311	GHA	CR	069 126 145 222 261 73 152 263 295 315.1	J	J1b8
312	GHA	CR	069 126 193 234 300 309 73 152 263 295 315.1	J	J1d1a
313	GHA	CR	069 126 193 234 300 309 73 152 263 295 315.1	J	J1d1a
316	GHA	CR	086 184 188.1 189d 192 223 278 294 309 390 73 146 152 195 263 315.1	J	J1d1a
343	GHA	CR	129 169 172 187 189 223 230 278 311 327 368 93 146 152 185 189 204 207 263 315.1	L	L0f
350	GHA	CR	189 192 223 278 294 309 390 73 143 146 152 195 263 309.1 315.1	L	L2a1a
356	GHA	CR	124 223 278 362 519 73 152 263 315.1	L	L3b1
359	GHA	CR	124 223 319 362 73 146 152 195 263 315.1	L	L3d1a1b
358	GHA	CR	124 223 256 368 73 152 263 315.1	L	L3d1d
361	GHA	CR	172 209 223 266 292 311 519 73 189 200 263	L	L3f1b2a
362	GHA	CR	209 223 292 294 311 327 465 519 532.1 73 152 263 315.1	L	L3f2
341	GHA	CR	145 223 362 390 502 519 535.1 73 152 204 207 263 315.1	N	N1b1
323	GHA	CR	126 185 355 362 519 58 64 146 183 263 315.1	R0	R0a1a
318	GHA	CR	126 294 296 519 60.1 73 263 315.1	T	T2
320	GHA	CR	172 266 292 294 519 73 263	T	T2c1

331	GHA	CR	270 296 73 150 263 315.1	U	U5b
89	NAF	CR	478 519 65 150 204 263 215.1	R0	H1av1
83	NAF	CR	519 152 200 263 309.1 315.1	R1	H
84	NAF	CR	124 224 311 519 73 263 315.1	R2	H
90	NAF	CR	519 146 263 315.1	R3	H1
91	NAF	CR	16519 146 263 315.1	R4	H1
92	NAF	CR	93 72 263 315.1	R5	H10f
88	NAF	CR	172 304 327 362 263 315.1 316	R6	H1bv1
93	NAF	CR	093 300 362 482 239 263 309.1 309.2 315.1	R7	H6b
375	NAF	CR	037 153 298 72 195 263 315.1	R8	HV0f
82	NAF	CR	311 391 263 315.1	R9	HV9
98	NAF	CR	224 311 519 73 263 315.1	K	K
99	NAF	CR	093 224 311 320 519 73 114 263 315.1	K	K1
103	NAF	CR	093 224 311 519 73 263	K	K1b1b
104	NAF	CR	224 311 519 73 146 152 199 263	K	K2a
105	NAF	CR	224 311 519 73 146 152 199 263 309.1 315.1	K	K2a
106	NAF	CR	224 311 519 73 146 152 199 263 309.1 315.1	K	K2a
110	NAF	CR	129 148 168 172 187 188 189 223 230 278 293 311 320 519 93 263 315.1	L	L0a1b1
112	NAF	CR	189 192 223 278 294 309 390 73 263 315.1	L	L2a1
115	NAF	CR	145 223 278 362 519 73263 315.1	L	L3b1a8
116	NAF	CR	186 189 192 223 256 284 311 73 189 263 315.1	L	L3h1b1
117	NAF	CR	179 223 243 265 284 311 320 519 73 152 189 194 195 215 263 315.1	L	L3h1b1a
118	NAF	CR	179 223 243 256 284 311 320 519 73 152 189 194 195 215 263 309.1 315.1	L	L3h1b1a
108	NAF	CR	129 185 189 223 249 311 519 73 195 200 263	M	M1b1a
78	NAF	CR	519 65 150 204 263 309.1 315.1	R0	R0
95	NAF	CR	192 270 526 73 263 315.1	U	U5a2
96	NAF	CR	192 256 270 526 73 263 315.1	U	U5a2
264	WAR	CR	172 209 223 266 292 311 519 73 189 200 263 291 309.1 315.1	L	L3f1b2a
179	WAR	CR	CRS 73 185 189 200 263 295 315.1	R0	H
180	WAR	CR	093 223 249 311 399 519 73 195 263 309.1 315.1	R0	H

181	WAR	CR	124 223 256 368 73 152 263	R0	H
182	WAR	CR	256 519 150 151 152 263 309.1 315.1 323.1	R0	H
183	WAR	CR	248 146 263 309.1 315.1	R0	H
184	WAR	CR	129 152 263 309.1 309.2 315.1	R0	H
195	WAR	CR	519 263 315.1	R0	H
194	WAR	CR	129 152 263 309.1 309.2 315.1	R0	H1
192	WAR	CR	129 519 150 263 309.1 315.1	R0	H1av1
193	WAR	CR	355 519 146 263 309.1 309.2 315.1	R0	H1m1
201	WAR	CR	069 126 261 73 185 189 263 315.1	R0	H7a1
163	WAR	CR	298 72 263 309.1 315.1	R0	HV0
164	WAR	CR	298 311 72 195 263 309.1 309.2 315.1	R0	HV0e
165	WAR	CR	067 343 519 263 309.1 315.1	R0	HV1
166	WAR	CR	067 343 519 263 309.1 315.1	R0	HV1
167	WAR	CR	067 274 295 200 263 309.1 315.1	R0	HV1b1
172	WAR	CR	256 352 60.1 263 315.1	R0	HV4a2a
175	WAR	CR	311 519 263 315.1	R0	HV9
176	WAR	CR	311 519 263 309.1 315.1	R0	HV9
220	WAR	CR	311 519 73 207 263 315.1	K	K
221	WAR	CR	224 260 311 519 44.1 73 146 247 263 315.1	K	K
240	WAR	CR	126 187 189 223 264 270 278 293 311 519 73 152 182 185 189195 247 263 315.1 357	L	L1b1a
241	WAR	CR	126 187 189 223 264 270 278 293 311 519 73 152 185 189 195 247 263 315.1 357	L	L1b1a
242	WAR	CR	126 186.1 189- 223 264 270 278 293 311 519 73 152 182 185 189 195 247 263 315.1	L	L1b1a
238	WAR	CR	223 278 294 296 390 73 143 145.1 146- 152 195 263 309.1 315.1	L	L1b1a18
239	WAR	CR	126 187 189 223 264 270 278 293 311 519 73 152 185 195 247 263 315.1	L	L1b1a18
247	WAR	CR	189 192 223 278 294 309 390 73 143 146 152 195 263 315.1	L	L2a
246	WAR	CR	093 189 192 223 278 294 309 343 390 73 143 146 152 195 263 309.1 315.1	L	L2a1
249	WAR	CR	124 223 73 263 315.1	L	L3
250	WAR	CR	124 223 278 362 519 73 263 315.1	L	L3b
252	WAR	CR	124 223 278 263 519 73 152 363 315.1	L	L3b
253	WAR	CR	124 223 278 362 519 73 152 217 263 315.1	L	L3b1b1

254	WAR	CR	093 124 223 278 362 519 73 152 217 263 315.1	L	L3b1b1
260	WAR	CR	124 223 73 152 263 315.1 523 525	L	L3d
261	WAR	CR	124 223 263 294 300 319 73 152 263 315.1	L	L3d1a1'2
265	WAR	CR	209 223 292 311 519 73 189 195 200 263 309.1 315.1	L	L3f1b
266	WAR	CR	209 223 292 311 519 73 150 189 263 309.1 315.1	L	L3f1b
267	WAR	CR	209 223 292 311 519 73 189 200 263 315.1	L	L3f1b
268	WAR	CR	176 188 209 223 234 73 263 315.1	L	L3f3b
269	WAR	CR	185.1 189- 192 223 256 284 311 73 189 263 315.1	L	L3h1b1
270	WAR	CR	188 189 223 274 292 293 311 316 335 355 362 399 519 73 146 152 195 244 263 291.1 315.1 340	L	L4b2b1
232	WAR	CR	129 185 189 223 249 311 73 195 200 263 309.1 309.2 309.3	M	M1b1a
227	WAR	CR	145 176 223 355 390 519 73 152 195 263 315.1	N	N1b1a7
229	WAR	CR	153 223 319 519 73 185 189 199 263 309.1 315.1 316	N	N2a
159	WAR	CR	092 126 224 362 263 315.1	R0	R0a
161	WAR	CR	092 126 224 362 57.1 64 263 309.1 309.2 315.1	R0	R0a
162	WAR	CR	126 57.1 64 263 309.1 309.2 315.1	R0	R0a
158	WAR	CR	126 355 362 58 64 146 152 263 309.1 315.1	R0	R0a1a
160	WAR	CR	126 304 362 57 1 64 263 315.1	R0	R0a2c
151	WAR	CR	126 294 296 519 73 263 309.1 315.1	T	T2
153	WAR	CR	126 294 296 304 519 73 151 256 263 309.1 315.1	T	T2b3c
152	WAR	CR	126 153 294 519 73 150 263 309.1 315.1	T	T2e
213	WAR	CR	126 294 311 343 390 519 73 150 195 263 315.1	U	U3a
214	WAR	CR	240 356 519 73 A 183 195 202 263 309.1 310	U	U4a2a
217	WAR	CR	184 318 381 519 73 152 263 309.1 315.1	U	U7
218	WAR	CR	126 148 309 318 519 73 146 151 152 195 263 315.1	U	U7a4
34	ZOU	CR	224 311 519 73 207 263 315.1	R0	H
35	ZOU	CR	124 223 73 152 263 315.1	R0	H
36	ZOU	CR	069 126 241 294 519 73 263 150 185.1 189 295 315.1	R0	H
37	ZOU	CR	224 311 519 73 207 263 315.1	R0	H
38	ZOU	CR	224 311 519 73 207 263 315.1	R0	H
43	ZOU	CR	069 126 189 261 73 185 189 263 315.1	R0	H

42	ZOU	CR	093 72 263 315.1	R0	H10f
41	ZOU	CR	519 65 150 204 263 315.1	R0	H1av1
31	ZOU	CR	311 391 199 263 315.1	R0	HV10
32	ZOU	CR	519 65 150 204 263 315.1	R0	HV12b
33	ZOU	CR	519 65 150 204 263 309.1 315.1	R0	HV12b
30	ZOU	CR	311 391 263 315.1	R0	HV9
1	ZOU	CR	069 126 73185 228 263 295 315.1 462 482 489	J	J1c1
2	ZOU	CR	069 126 73 185 228 263 295 315.1 462 482 489	J	J1c1
3	ZOU	CR	069 126 73 185 228 263 295 315.1 462 482 489	J	J1c1
4	ZOU	CR	069 126 73 185 228 263 295 315.1 462 482 489	J	J1c1
5	ZOU	CR	069 126 73 185 228 263 295 315.1 462 482 489	J	J1c1
6	ZOU	CR	069 126 73 185 228 263 295 315.1 462 482 489	J	J1c1
7	ZOU	CR	069 126 73 185 228 263 295 315.1 462 482 489	J	J1c1
8	ZOU	CR	069 126 73 185 228 263 295 315.1 462 482 489	J	J1c1
9	ZOU	CR	069 126 73 185 228 263 295 315.1 462 482 489	J	J1c1
59	ZOU	CR	223 1 24 311 519 73 263 315.1	K	K
60	ZOU	CR	224 311 320 519 73 114 263 319.1 315.1	K	K
61	ZOU	CR	311 320 73 263 315.1	K	K
62	ZOU	CR	224 311 320 519 73 114 263 30 9.1315.1	K	K
63	ZOU	CR	224 311 519 73 263 315.1	K	K
64	ZOU	CR	224 311 519 73 263 315.1	K	K
65	ZOU	CR	093 224 311 519 73 207 263 315.1	K	K1b1b
66	ZOU	CR	093 224 311 519 73 195 263 315.1	K	K1b1b
67	ZOU	CR	093 224 311 519 73 263 315.1	K	K1b1b
75	ZOU	CR	189 223 245 294 309 386 390 73 143 146 195 263 315.1	L	L2a
76	ZOU	CR	086 184 192 223 278 294 309 390 73 146 152 263 315.1	L	L2a
74	ZOU	CR	189 223 278 294 309 390 73 143 146 152 195 263 309.1 315.1	L	L2a1
77	ZOU	CR	124 223 73 152 263 315.1	L	L3d
71	ZOU	CR	129 185 189dt 223 249 311 519 73 195 200 263 315.1	M	M1b1a
51	ZOU	CR	226 192 265 270 526 73 263 315.1	U	U5a2

52	ZOU	CR	092 172 219 278 73 263 315.1	U	U6a
53	ZOU	CR	092 172 219 278 73 263 315.1	U	U6a
54	ZOU	CR	092 172 219 223 278 73 152 263 315.1	U	U6a
127	AFL	FL	64, 93, 152, 185, 189, 236, 247, 263, 315+C, 523-524d, 750, 769, 825A, 1018, 1048, 1438, 2245, 2706, 2758, 2885, 3516A, 3594, 3866, 4104, 4312, 4586, 4769, 5096, 5231, 5442, 5460, 5603, 6185, 7028, 7256, 7521, 8428, 8468, 8566, 8655, 8701, 8860, 9042, 9305C, 9347, 9540, 9755, 9818, 10398, 10589, 10664, 10688, 10810, 10873, 10915, 11167, 11176, 11641, 11719, 11914, 12007, 12705, 12720, 13105, 13276, 13506, 13650, 13708, 13988G, 13993+G, 14308, 14766, 15136, 15326, 15431, 16148, 16168, 16172, 16187, 16223, 16230, 16311, 16320, 16519	L	L0a1a
128	AFL	FL	64, 93, 152, 185, 189, 236, 247, 263, 315+C, 523-524d, 750, 769, 825A, 1018, 1048, 1438, 2245, 2706, 2758, 2885, 3516A, 3594, 3866, 4104, 4312, 4586, 4769, 5096, 5231, 5442, 5460, 5603, 6185, 7028, 7256, 7521, 8428, 8468, 8566, 8655, 8701, 8860, 9042, 9305C, 9347, 9540, 9755, 9818, 10398, 10589, 10664, 10688, 10810, 10873, 10915, 11167, 11176, 11641, 11719, 11914, 12007, 12705, 12720, 13105, 13276, 13506, 13650, 13708, 13988G, 13993+G, 14308, 14766, 15136, 15326, 15431, 16148, 16168, 16172, 16187, 16223, 16230, 16311, 16320, 16519	L	L0a1a
131	AFL	FL	73, 152, 185T, 189, 195, 247, 263, 315+C, 357, 501, 523-524d, 709, 750, 769, 825A, 1018, 1738, 2352, 2706, 2758, 2768, 2885, 3308, 3594, 3666, 3693, 4104, 4769, 5036, 5046, 5393, 5655, 6548, 6827, 6989, 7028, 7055, 7146, 7256, 7389, 7521, 7867, 8248, 8468, 8655, 8701, 8860, 9079, 9540, 10398, 10688, 10810, 10873, 11719, 12519, 12609, 12696, 12705, 13105, 13506, 13650, 13789, 13880A, 14178, 14203, 14560, 14766, 14769, 15115, 15326, 16126, 16187, 16189, 16223, 16264, 16270, 16278, 16292, 16293, 16311, 16519	L	L1b1a18
132	AFL	FL	73, 152, 185T, 189, 195, 247, 263, 315+C, 357, 501, 523-524d, 709, 750, 769, 825A, 1018, 1738, 2352, 2706, 2758, 2768, 2885, 3308, 3594, 3666, 3693, 4104, 4769, 5036, 5046, 5393, 5655, 6548, 6827, 6989, 7028, 7055, 7146, 7256, 7389, 7521, 7867, 8248, 8468, 8655, 8701, 8860, 9079, 9540, 10398, 10688, 10810, 10873, 11719, 12519, 12609, 12696, 12705, 13105, 13506, 13650, 13789, 13880A, 14178, 14203, 14560, 14766, 14769, 15115, 15326, 16126, 16187, 16189, 16223, 16264, 16270, 16278, 16292, 16293, 16311, 16519	L	L1b1a18
135	AFL	FL	73, 263, 309+C, 315+C, 372, 523-524d, 750, 1438, 2706, 3450, 4769, 5773, 5853, 6221, 7028, 8347, 8701, 8860, 9449, 9540, 10086, 10373, 10398, 10873, 11002, 11719, 12705, 13105, 13988G, 13993+G, 14766, 15301, 15326, 15824, 15944d, 16124, 16223, 16278, 16519	L	L3b1a
136	AFL	FL	73, 263, 309+C, 315+C, 372, 523-524d, 750, 1438, 2706, 3450, 4769, 5773, 5853, 6221, 7028, 8347, 8701, 8860, 9449, 9540, 10086, 10373, 10398, 10873, 11002, 11719, 12705, 13105, 13988G, 13993+G, 14766, 15301, 15326, 15824, 15944d, 16124, 16223, 16278, 16519	L	L3b1a
139	AFL	FL	73, 150, 152, 189, 193, 263, 315+C, 750, 1428, 1438, 2352, 2706, 4769, 6221, 7028, 8701, 8860, 9079, 9449, 9540, 10086, 10373, 10398, 10873, 11719, 12705, 13105, 13708, 13988G, 13993+G, 14152, 14212, 14766, 15301, 15311, 15326, 15664, 15670, 15824, 15942, 16093, 16223, 16278, 16362, 16519	L	L3b1b1
140	AFL	FL	73, 150, 152, 189, 193, 263, 315+C, 750, 1428, 1438, 2352, 2706, 4769, 6221, 7028, 8701, 8860, 9079, 9449, 9540, 10086, 10373, 10398, 10873, 11719, 12705, 13105, 13708, 13988G, 13993+G, 14152, 14212, 14766, 15301, 15311, 15326, 15664, 15670, 15824, 15942, 16093, 16223, 16278, 16362, 16519	L	L3b1b1
141	AFL	FL	73, 152, 195, 262, 263, 315+C, 523-524d, 750, 921, 1438, 2706, 4769, 5147, 7028, 7424, 8618, 8701, 8860, 9230, 9540, 10398, 10873, 11617, 11719, 12705, 13105, 13886, 13988G, 14180, 14284, 14766, 15301, 15326, 15434A, 15799, 16124, 16223, 16240T, 16362, 16519	L	L3d5
142	AFL	FL	73, 152, 195, 262, 263, 315+C, 523-524d, 750, 921, 1438, 2706, 4769, 5147, 7028, 7424, 8618, 8701, 8860, 9230, 9540, 10398, 10873, 11617, 11719, 12705, 13105, 13886, 13988G, 14180, 14284, 14766, 15301, 15326, 15434A, 15799, 16124, 16223, 16240T, 16362, 16519	L	L3d5
143	AFL	FL	73, 152, 195, 262, 263, 315+C, 523-524d, 750, 921, 1438, 2706, 4769, 5147, 7028, 7424, 8618, 8701, 8860, 9230, 9540, 10398, 10873, 11617, 11719, 12705, 13105, 13886, 13988G, 14180, 14284, 14766, 15301, 15326, 15434A, 15799, 16124, 16223, 16240T, 16362, 16519	L	L3d5
144	AFL	FL	73, 150, 189, 193, 200, 263, 309+C, 315+C, 750, 1438, 2352, 2706, 4769, 6221, 6587, 7028, 8701, 8860, 9296G, 9540, 10398, 10819, 10873, 11719, 12346, 12705, 13708, 14152, 14212, 14766, 15301, 15326, 15670, 15942, 16223, 16327	L	L3e1
145	AFL	FL	73, 150, 195, 236, 263, 315+C, 523-524d, 1438, 2000, 2352, 2706, 4769, 5262, 5951, 6071, 7028, 7055, 7146, 7256, 7389, 7521, 8027, 8468, 8655, 8701, 8860, 8937, 9072, 9540, 10321, 10398, 10586, 10688, 10810, 10873, 11719, 11899, 11914, 12705, 12810, 13105, 13485, 13506, 13650, 14212, 14766, 15301, 15326, 15812, 16093, 16223, 16265T, 16519	L	L3e3'4
146	AFL	FL	73, 150, 195, 236, 263, 315+C, 523-524d, 1438, 2000, 2352, 2706, 4769, 5262, 5951, 6071, 7028, 7055, 7146, 7256, 7389, 7521, 8027, 8468, 8655, 8701, 8860, 8937, 9072, 9540, 10321, 10398, 10586, 10688, 10810, 10873, 11719, 11899, 11914, 12705, 12810, 13105, 13485, 13506, 13650, 14212, 14766, 15301, 15326, 15812, 16093, 16223, 16265T, 16519	L	L3e3'4
149	AFL	FL	73, 150, 195, 236, 263, 315+C, 523-524d, 1438, 2000, 2352, 2706, 4769, 5262, 5951, 6071, 7028, 7055, 7146, 7256, 7389, 7521, 8027, 8468, 8655, 8701, 8860, 8937, 9072, 9540, 10321, 10398, 10586, 10688, 10810, 10873, 11719, 11899, 11914, 12705, 12810, 13105, 13485, 13506, 13650, 14212, 14766, 15301, 15326, 15812, 16093, 16223, 16265T, 16519	L	L3e3'4
126	AFL	FL	73, 195, 263, 309+C, 315+C, 489, 750, 1438, 1842, 2706, 4769, 5250, 5351, 5484, 6446, 6680, 7028, 8701, 8860, 9540, 10398, 10400, 10873, 10895, 11719, 12403, 12705, 12950C, 13111, 14110, 14766, 14783, 15043, 15301, 15326, 16129, 16183C, 16189, 16223, 16249, 16311, 16399, 16519	M	M1b2
123	AFL	FL	73, 152, 204, 207, 263, 315+C, 750, 1438, 1598, 1703, 1719, 2639, 2706, 3921A, 4769, 4904, 4960, 5471, 7028, 8251, 8472, 8836, 8860, 9335, 10238, 11362, 11719, 12501, 12705, 12822, 14766, 15326, 16145, 16176G, 16223, 16362, 16390, 16502, 16519, 16535+T	N	N1b1a2
124	AFL	FL	73, 152, 204, 207, 263, 315+C, 750, 1438, 1598, 1703, 1719, 2639, 2706, 3921A, 4769, 4904, 4960, 5471, 7028, 8251, 8472, 8836, 8860, 9335, 10238, 11362, 11719, 12501, 12705, 12822, 14766, 15326, 16145, 16176G, 16223, 16362, 16390, 16502, 16519, 16535+T	N	N1b1a2

282	EAR	FL	263, 750, 1438, 3447C, 3468C, 3473, 3475C, 4769, 8512C, 8860, 11914, 13993+G, 15326, 16519	R0	H
286	EAR	FL	263, 750, 1438, 3010, 3447C, 3468C, 3473, 4769, 5363, 8860, 9797, 13993+G, 15326, 16519	R0	H1
290	EAR	FL	263, 750, 1438, 3834C, 4769, 8860, 11839, 12358, 13993+G, 15326, 16519	R0	H100
284	EAR	FL	73, 152, 263, 750, 1438, 3010, 4769, 8860, 10289, 11864, 13988G, 13993+G, 14860G, 14862G, 15326, 16214, 16519	R0	H1bb
285	EAR	FL	195, 263, 750, 1438, 3010, 4769, 5460, 7177, 8860, 13988G, 13993+G, 15326, 16519	R0	H1e
365	EAR	FL	195, 263, 750, 1438, 3010, 4769, 5460, 7177, 8860, 13988G, 13993+G, 15326, 16519	R0	H1e
366	EAR	FL	73, 150, 189, 193, 200, 263, 295, 309+C, 315+C, 489, 750, 1438, 1733, 2352, 2706, 3010, 4216, 4769, 5182, 5267, 6221, 7028, 8269, 8860, 10398, 11251, 11719, 12612, 13708, 13988G, 13993+G, 14152, 14212, 14766, 15301, 15326, 15452A, 15670, 15942, 16069, 16126, 16145, 16222, 16223, 16261, 16327	R0	H1e
271	EAR	FL	73, 263, 271, 295, 309+C, 315+C, 462, 489, 750, 1438, 2706, 3010, 3736, 4216, 4769, 5460, 8860, 9083, 11251, 11719, 12612, 13708, 13879, 13993+G, 14766, 15326, 15452A, 16069, 16126, 16145, 16222, 16235, 16261, 16294, 16519	J	J1b1b2
368	EAR	FL	73, 150, 189, 263, 309+C, 315+C, 497, 573+XC, 750, 1189, 1393, 1438, 1811, 2706, 3480, 4769, 5581, 7028, 7897, 8860, 9055, 9698, 10398, 10550, 11299, 11467, 11719, 12308, 12372, 12523, 14167, 14766, 14798, 15326, 15398, 16093, 16224, 16311	J	J1b2
274	EAR	FL	73, 152, 263, 295, 315+C, 462, 489, 750, 1007, 1438, 2706, 3010, 4048, 4216, 4769, 7805, 8407A, 8557, 8860, 11467, 11719, 12308, 12372, 12501, 12618, 13440, 13651, 13708, 13988G, 14766, 15326, 15452A, 16069, 16126, 16193, 16234, 16300, 16309	J	J1d1a
275	EAR	FL	73, 152, 263, 295, 315+C, 462, 489, 750, 1007, 1438, 2706, 3010, 4048, 4216, 4769, 7391, 7805, 8407A, 8557, 8860, 11467, 11719, 12308, 12372, 12501, 12618, 13440, 13651, 13708, 13988G, 14766, 15326, 15452A, 16069, 16126, 16193, 16234, 16300, 16309	J	J1d1a
372	EAR	FL	73, 150, 189, 263, 309+C, 315+C, 497, 573+XC, 750, 1189, 1393, 1438, 1811, 2706, 3480, 4769, 5581, 7028, 7897, 8860, 9055, 9698, 10398, 10550, 11299, 11467, 11719, 12308, 12372, 12523, 14167, 14766, 14798, 15326, 15398, 16093, 16224	K	K1a
291	EAR	FL	73, 114, 263, 315+C, 497, 750, 1189, 1438, 1811, 2706, 3480, 4769, 5583, 7028, 8224, 8860, 9055, 9698, 10398, 10550, 11299, 11467, 11470, 11719, 11914, 12007, 12308, 12372, 12555, 13993+G, 14167, 14766, 14798, 14860G, 15326, 15412, 15924, 16093, 16224, 16311, 16519	K	K1a1b1g
297	EAR	FL	73, 152, 185T, 189, 195, 247, 263, 315+C, 357, 501, 523-524d, 709, 750, 769, 825A, 1018, 1738, 2352, 2706, 2758, 2768, 2885, 3308, 3594, 3666, 3693, 4104, 4769, 5036, 5046, 5393, 5655, 6548, 6827, 6989, 7028, 7055, 7146, 7256, 7389, 7521, 7867, 8248, 8468, 8655, 8701, 8860, 9079, 9540, 10398, 10688, 10810, 10873, 11719, 12519, 12609, 12696, 12705, 13105, 13506, 13650, 13789, 13880A, 14178, 14203, 14560, 14766, 14769, 15115, 15326, 16126, 16187, 16189, 16223, 16264, 16270, 16278, 16292, 16293, 16311, 16519	L	L1b1a18
300	EAR	FL	73, 263, 309+C, 315+C, 372, 523-524d, 750, 1438, 2706, 3450, 4769, 5773, 5853, 6221, 7028, 8347, 8701, 8860, 9449, 9540, 10086, 10373, 10398, 10873, 11002, 11719, 12705, 13105, 13988G, 13993+G, 14766, 15301, 15326, 15824, 15944d, 16124, 16223, 16278, 16519	L	L3b1a
302	EAR	FL	73, 150, 189, 193, 200, 263, 309+C, 315+C, 750, 1438, 2352, 2706, 4769, 6221, 7028, 7175, 7256, 7274, 7521, 7771, 8206, 8701, 8860, 9221, 9540, 10115, 10398, 10873, 11719, 11914, 11944, 12693, 12705, 13708, 13988G, 13993+G, 14152, 14212, 14766, 15301, 15326, 15670, 15942, 16223, 16327	L	L3e1
303	EAR	FL	73, 150, 189, 193, 200, 263, 309+C, 315+C, 750, 1438, 2352, 2706, 4769, 6221, 6587, 7028, 8701, 8860, 9296G, 9540, 10398, 10819, 10873, 11719, 12346, 12705, 13708, 14152, 14212, 14766, 15301, 15326, 15670, 15942, 16223, 16327	L	L3e1
373	EAR	FL	73, 195, 198, 263, 315+C, 325, 523-524d, 750, 769, 1018, 2706, 3357, 4769, 5201, 5460, 6167, 7028, 7376, 7762, 7775, 8473, 8631, 8701, 8860, 9452, 9540, 10373, 10398, 10873, 11253, 11344, 11485, 11653, 11719, 12414, 12705, 13174, 14000A, 14302, 14515, 14766, 15301, 15326, 15409, 16093, 16207C, 16223, 16260, 16311, 16362, 16519	L	L4a1a
294	EAR	FL	73, 200, 263, 315+C, 750, 1438, 2706, 4769, 7028, 8251, 8472, 8836, 8860, 9335, 10238, 11362, 11719, 12501, 12705, 12822, 13708, 13988G, 13993+G, 14766, 15326, 15670, 16223, 16326d, 16519	N	N1
295	EAR	FL	73, 152, 204, 207, 263, 315+C, 750, 1438, 1598, 1703, 1719, 2639, 2706, 3921A, 4769, 4904, 4960, 5471, 7028, 8251, 8472, 8836, 8860, 9335, 10238, 11362, 11719, 12501, 12705, 12822, 14766, 15326, 16145, 16176G, 16223, 16362, 16390, 16502, 16519, 16535+T	N	N1b1a2
280	EAR	FL	73, 263, 315+C, 709, 750, 1438, 1888, 2706, 4216, 4769, 4917, 7028, 8260, 8697, 8860, 10463, 11251, 11393A, 11569, 11719, 11812, 13368, 14233, 14766, 14905, 15326, 15452A, 15607, 15724, 15928, 16126, 16294, 16296, 16519	T	T2
281	EAR	FL	73, 152, 263, 315+C, 533, 573+XC, 709, 750, 1438, 1842, 1888, 2706, 4216, 4769, 4917, 6261, 7028, 8455, 8697, 8860, 10463, 10822, 11251, 11719, 11812, 13368, 13770, 13928C, 13973T, 14233, 14766, 14905, 15326, 15452A, 15607, 15691, 15928, 16126, 16172, 16266, 16292, 16294, 16519	T	T2c1a
328	GHA	FL	263, 750, 1438, 1700, 4769, 8860, 9438, 9554, 13988G, 13993+G, 15326, 16145, 16519	R0	H
329	GHA	FL	263, 750, 1438, 1700, 4769, 8860, 9438, 9554, 13988G, 13993+G, 15326, 16145, 16519	R0	H
326	GHA	FL	73, 263, 750, 1438, 1810, 2452, 2706T, 3010, 3405C, 3428C, 3441C, 3447C, 3460C, 3468C, 3473, 3492C, 3499C, 3575, 3607C, 3615M, 3743A, 4769, 5460, 8512, 8860, 13988G, 13993+G, 15326, 16519	R0	H1e1

309	GHA	FL	73, 263, 271, 295, 309+C, 315+C, 462, 489, 750, 1438, 2706, 3010, 3736, 4216, 4769, 5460, 7391, 8860, 9083, 11251, 11719, 12612, 13708, 13879, 13993+G, 14766, 15326, 15452A, 16069, 16126, 16145, 16222, 16235, 16261, 16294, 16519	J	J1b1b2
310	GHA	FL	73, 263, 271, 295, 309+C, 315+C, 462, 489, 750, 1438, 2706, 3010, 3736, 4216, 4769, 5460, 8860, 9083, 11251, 11719, 12612, 13708, 13879, 13993+G, 14766, 15326, 15452A, 16069, 16126, 16145, 16222, 16235, 16261, 16294, 16519	J	J1b1b2
314	GHA	FL	73, 152, 263, 295, 315+C, 462, 489, 750, 1007, 1438, 2706, 3010, 4048, 4216, 4769, 7391, 7805, 8407A, 8557, 8860, 11467, 11719, 12308, 12372, 12501, 12618, 13440, 13651, 13708, 13988G, 14766, 15326, 15452A, 16069, 16126, 16193, 16234, 16300, 16309	J	J1d1a1
315	GHA	FL	73, 152, 263, 295, 315+C, 462, 489, 750, 1007, 1438, 2706, 3010, 4048, 4216, 4769, 7391, 7805, 8407A, 8557, 8860, 11467, 11719, 12308, 12372, 12501, 12618, 13440, 13651, 13708, 13988G, 14766, 15326, 15452A, 16069, 16126, 16193, 16234, 16300, 16309	J	J1d1a1
317	GHA	FL	73, 152, 263, 295, 315+C, 462, 489, 750, 1007, 1438, 2706, 3010, 4048, 4216, 4769, 7391, 7805, 8407A, 8557, 8860, 11467, 11719, 12308, 12372, 12501, 12618, 13440, 13651, 13708, 13988G, 14766, 15326, 15452A, 16069, 16126, 16193, 16234, 16300, 16309	J	J1d1a1
370	GHA	FL	73, 195, 263, 309+C, 315+C, 489, 750, 813, 1438, 1503, 2706, 4769, 5253, 6446, 6671, 6680, 7028, 8701, 8860, 9380, 9540, 10398, 10400, 10873, 11719, 12403, 12414, 12705, 12950C, 13637, 14110, 14766, 14783, 15043, 15301, 15326, 16183C, 16189, 16249, 16265C, 16311, 16519	K	K1a
338	GHA	FL	73, 114, 263, 315+C, 497, 750, 1189, 1438, 1811, 2706, 3480, 4769, 5583, 7028, 8224, 8860, 9055, 9698, 10398, 10550, 11299, 11467, 11470, 11719, 11914, 12007, 12308, 12372, 12555, 14167, 14766, 14798, 15326, 15412, 15924, 16093, 16224, 16311, 16519	K	K1a1b1g
339	GHA	FL	73, 263, 315+C, 497, 750, 1189, 1438, 1811, 2706, 3480, 4769, 5583, 7028, 8224, 8860, 9055, 9698, 10398, 10550, 11299, 11467, 11470, 11719, 11914, 12007, 12308, 12372, 12555, 14167, 14766, 14798, 15326, 15412, 15924, 16224, 16311, 16519	K	K1a1b1g
344	GHA	FL	73, 152, 182, 185T, 247, 263, 315+C, 357, 523-524d, 709, 750, 769, 825A, 1018, 2352, 2706, 2758, 2768, 2885, 3308, 3594, 3666, 3693, 4104, 4769, 5036, 5046, 5393, 5655, 6548, 6827, 6851, 6989, 7028, 7055, 7146, 7256, 7389, 7521, 7867, 8248, 8468, 8655, 8701, 8860, 9540, 10398, 10688, 10810, 10873, 11719, 12236C, 12519, 12705, 13105, 13494, 13506, 13708, 13988G, 13993+G, 14178, 14203, 14560, 14766, 14769, 15115, 15326, 15787, 16126, 16187, 16189, 16223, 16264, 16270, 16278, 16311	L	L1b1a
345	GHA	FL	73, 146, 152, 195, 263, 315+C, 750, 769, 1018, 1438, 2416, 2487, 2706, 2789, 3594, 4104, 4769, 5894C, 7028, 7175, 7256, 7274, 7521, 7702, 7771, 8206, 8496, 8701, 8860, 9221, 9540, 10115, 10398, 10873, 11719, 11914, 11944, 12693, 12705, 13590, 13650, 13803, 13988G, 13993+G, 14566, 14766, 15301, 15326, 15784, 16086, 16184, 16189, 16192, 16223, 16278, 16294, 16309, 16390	L	L2a1
346	GHA	FL	73, 146, 152, 195, 263, 315+C, 750, 769, 1018, 1438, 2416, 2487, 2706, 2789, 3594, 4104, 4769, 5894C, 7028, 7175, 7256, 7274, 7521, 7702, 7771, 8206, 8496, 8701, 8860, 9221, 9540, 10115, 10398, 10873, 11719, 11914, 11944, 12693, 12705, 13590, 13650, 13803, 13988G, 13993+G, 14566, 14766, 15301, 15326, 15784, 16086, 16184, 16189, 16192, 16223, 16278, 16294, 16309, 16390	L	L2a1b
347	GHA	FL	73, 146, 152, 195, 263, 309+CC, 315+C, 750, 769, 1018, 1438, 2416, 2706, 2789, 3594, 4104, 4317, 4769, 7028, 7175, 7256, 7274, 7521, 7771, 8206, 8701, 8860, 9221, 9540, 10115, 10143, 10398, 10873, 11656, 11719, 11914, 11944, 12693, 12705, 13590, 13650, 13803, 13988G, 13993+G, 14566, 14766, 15301, 15326, 15784, 16189, 16192, 16223, 16278, 16294, 16309, 16390	L	L2a1b
348	GHA	FL	73, 146, 152, 195, 263, 309+CC, 315+C, 750, 769, 1018, 1438, 2416, 2706, 2789, 3594, 4104, 4317, 4769, 7028, 7175, 7256, 7274, 7521, 7771, 8206, 8701, 8860, 9221, 9540, 10115, 10143, 10398, 10873, 11656, 11719, 11914, 11944, 12693, 12705, 13590, 13650, 13803, 13988G, 13993+G, 14566, 14766, 15301, 15326, 15784, 16184, 16189, 16192, 16223, 16278, 16294, 16309, 16390	L	L2a1b
349	GHA	FL	73, 146, 152, 195, 263, 309+CC, 315+C, 750, 769, 1018, 1438, 2416, 2706, 2789, 3594, 4104, 4317, 4769, 7028, 7175, 7256, 7274, 7521, 7771, 8206, 8701, 8860, 9221, 9540, 10115, 10143, 10398, 10873, 11656, 11719, 11914, 11944, 12693, 12705, 13590, 13650, 13803, 13988G, 13993+G, 14566, 14766, 15301, 15326, 15784, 16189, 16192, 16223, 16278, 16294, 16309, 16390	L	L2a1b
374	GHA	FL	73, 143, 146, 152, 195, 263, 309+C, 315+C, 750, 769, 1018, 1438, 2416, 2706, 2789, 3594, 4104, 4769, 6899, 7028, 7175, 7256, 7274, 7521, 7771, 8206, 8701, 8860, 9221, 9540, 10115, 10398, 10873, 11719, 11914, 11944, 12693, 12705, 13590, 13650, 13803, 13884, 14566, 14766, 15301, 15326, 15784, 16189d, 16192+TT, 16223, 16278, 16294, 16309, 16390	L	L2a1m
351	GHA	FL	73, 146, 150, 152, 182, 186, 195, 198, 204, 263, 315+C, 750, 769, 796, 1018, 1438, 1442, 1706, 2332, 2358, 2416, 2706, 3594, 4104, 4158, 4370, 4767, 4769, 5027, 5331A, 5814, 6614, 6713, 6806, 7028, 7256, 7521, 7624A, 8080, 8206, 8387, 8503, 8701, 8860, 9221, 9540, 10115, 10398, 10873, 11023C, 11719, 11944, 12705, 12948, 13590, 13650, 13988G, 13993+G, 14059, 14766, 15110, 15217, 15301, 15326, 16114A, 16129, 16189, 16193+N, 16213, 16223, 16278, 16309, 16357, 16390	L	L2b2
360	GHA	FL	73, 152, 263, 309+C, 315+C, 750, 1438, 2706, 4769, 5601, 7028, 8701, 8860, 9540, 9950, 10398, 10873, 11440, 11719, 12705, 14766, 15301, 15326, 16223, 16368Y, 16519	L	L3
352	GHA	FL	73, 263, 309+C, 315+C, 372, 523-524d, 750, 1438, 2706, 3450, 4769, 5773, 5853, 6221, 7028, 8347, 8701, 8860, 9449, 9540, 10086, 10373, 10398, 10873, 11002, 11719, 12705, 13105, 13988G, 13993+G, 14766, 15301, 15326, 15824, 15944d, 16124, 16223, 16278, 16519	L	L3b1a
353	GHA	FL	73, 263, 309+C, 315+C, 523-524d, 750, 1438, 1710C, 2706, 3450, 4769, 5773, 6221, 7028, 8701, 8860, 9449, 9540, 9605, 10086, 10310, 10373, 10398, 10873, 11002, 11719, 12705, 13105, 13914A, 14094, 14766, 15301, 15311, 15326, 15670, 15824, 15944d, 16124, 16223, 16278, 16362, 16519, 16549+C	L	L3b1a4
354	GHA	FL	73, 263, 309+C, 315+C, 523-524d, 750, 1438, 1710C, 2706, 3450, 4769, 5773, 6221, 7028, 8701, 8860, 9449, 9540, 9605, 10086, 10310, 10373, 10398, 10873, 11002, 11719, 12705, 13105, 13914A, 14094, 14766, 15301, 15311, 15326, 15670, 15824, 15944d, 16124, 16223, 16278, 16362, 16519, 16549+C	L	L3b1a4

355	GHA	FL	73, 263, 309+C, 315+C, 523-524d, 750, 1438, 2706, 3420, 3450, 4769, 5250, 5773, 5955, 6071, 6221, 7028, 8701, 8860, 9449, 9540, 10086, 10398, 10640, 10873, 11719, 12705, 13914A, 14766, 15301, 15311, 15326, 15824, 16124, 16183C, 16189, 16223, 16278, 16362, 16527	L	L3b2a
357	GHA	FL	73, 152, 263, 309+C, 315+C, 523-524d, 750, 921, 1438, 2706, 4769, 5147, 5688G, 6150, 6524A, 6680, 7028, 7424, 7765, 8618, 8701, 8709, 8860, 9151, 9540, 10398, 10873, 11719, 12705, 13105, 13988G, 14284, 14766, 15301, 15326, 15942, 16223, 16256, 16368	L	L3d1d
342	GHA	FL	73, 195, 263, 309+C, 315+C, 489, 750, 813, 1438, 2706, 3705, 4769, 6446, 6671, 6680, 7028, 8701, 8860, 9540, 10398, 10400, 10873, 11719, 12346, 12403, 12705, 12950C, 13988G, 13993+G, 14110, 14766, 14783, 15043, 15301, 15326, 16129, 16189, 16193+C, 16223, 16234, 16249, 16260, 16311, 16359, 16519	M	M1a1
340	GHA	FL	73, 146, 153, 189C, 199, 263, 315+C, 750, 1438, 2706, 4769, 6221, 6371A, 7028, 7258, 8860, 11719, 12705, 13785, 13993+G, 14323, 15326, 16223, 16311, 16390	N	N
321	GHA	FL	58, 64, 146, 183, 263, 309+C, 315+C, 750, 827, 1438, 2442, 2706, 3438, 3847, 4769, 5120, 5333, 5913, 7028, 7325, 8292, 8860, 11761, 13188, 13695, 13988G, 13993+G, 14766, 15326, 16126, 16185, 16355, 16362, 16519	R0	R0a1a4
322	GHA	FL	58, 64, 146, 183, 263, 309+C, 315+C, 750, 827, 1438, 2442, 2706, 3438, 3847, 4769, 5120, 5333, 5913, 7028, 7325, 8292, 8860, 11761, 13188, 13695, 13988G, 13993+G, 14766, 15326, 16126, 16185, 16355, 16362, 16519	R0	R0a1a4
319	GHA	FL	73, 152, 263, 315+C, 533, 573+XC, 709, 750, 1438, 1842, 1888, 2706, 4216, 4769, 4917, 6261, 7028, 8455, 8697, 8860, 10463, 10822, 11251, 11719, 11812, 13368, 13770, 13928C, 13973T, 14233, 14766, 14905, 15326, 15452A, 15607, 15691, 15928, 16126, 16172, 16266, 16292, 16294, 16519	T	T2c1a
330	GHA	FL	73, 93, 150, 195, 263, 309+C, 315+C, 750, 1438, 2706, 3197, 3398, 4769, 5656, 7028, 7768, 8860, 9477, 11467, 11719, 12308, 12372, 13617, 13993+G, 14182, 14766, 15122, 15326, 16189, 16193, 16270, 16274, 16465, 16519	U Incl. (K)	U5b1e
332	GHA	FL	73, 263, 315+C, 498, 750, 1438, 2706, 3348, 4769, 5471, 7028, 7391, 7805, 8407, 8557, 8860, 11467, 11719, 12308, 12372, 12501, 12618, 13440, 13988G, 13993+G, 14179, 14766, 15326, 16092, 16172, 16189, 16219, 16278	U	U6a
333	GHA	FL	73, 103, 143, 263, 309+C, 315+C, 1438, 2706, 2775, 3348, 4769, 5813, 7028, 7805, 7813, 8282, 8860, 9752, 10172, 11467, 11539, 11719, 12308, 12372, 13781, 13988G, 13993+G, 14179, 14766, 15326, 16172, 16189, 16219, 16278	U	U6a8a
334	GHA	FL	73, 103, 143, 263, 309+C, 315+C, 1438, 2706, 2775, 3348, 4769, 5813, 7028, 7805, 7813, 8282, 8860, 9752, 10172, 11467, 11539, 11719, 12308, 12372, 13781, 13988G, 13993+G, 14179, 14766, 15326, 16172, 16189, 16219, 16278	U	U6a8a
335	GHA	FL	73, 103, 143, 263, 309+C, 315+C, 1438, 2706, 2775, 3348, 4769, 5813, 7028, 7805, 7813, 8282, 8860, 9752, 10172, 11467, 11539, 11719, 12308, 12372, 13781, 13988G, 13993+G, 14179, 14766, 15326, 16172, 16189, 16219, 16278	U	U6a8a
336	GHA	FL	73, 235, 263, 309+C, 315+C, 750, 1438, 2706, 3348, 4769, 7028, 8860, 9438, 11467, 11719, 12308, 12372, 14766, 15326, 16172, 16219, 16278, 16311	U	U6b3a
337	GHA	FL	73, 235, 263, 309+C, 315+C, 750, 1438, 2706, 3348, 4769, 7028, 8860, 9438, 11467, 11719, 12308, 12372, 14766, 15326, 16172, 16219, 16278, 16311	U	U6b3a
87	NAF	FL	195, 263, 750, 1438, 3010, 4769, 5460, 8860, 11084, 13988G, 13993+G, 15326, 16519	R0	H1e, H1bk
85	NAF	FL	72, 263, 750, 1438, 4769, 8454, 8860, 9230, 13988G, 13993+G, 15326, 15884, 16093, 16214	R0	H
86	NAF	FL	195, 263, 750, 1438, 3010, 4769, 5460, 8860, 11084, 13988G, 13993+G, 15326, 16519	R0	H1ap1
80	NAF	FL	199, 263, 315+C, 750, 1438, 2706, 4769, 6221, 6371, 7028, 7258, 8860, 11719, 12705, 14323, 15326, 16311, 16391	R0	HV
81	NAF	FL	199, 263, 315+C, 750, 1438, 2706, 4769, 6221, 6371, 7028, 7258, 8860, 11719, 12705, 14323, 15326, 16311, 16391	R0	HV
100	NAF	FL	73, 114, 263, 309+C, 315+C, 497, 524+AC, 750, 1189, 1438, 1811, 2706, 3480, 4769, 7028, 7664, 8419, 8860, 9055, 9698, 10398, 10550, 11299, 11467, 11719, 11914, 12308, 12372, 12651, 13105, 13993+G, 14167, 14766, 14798, 15326, 16093, 16224, 16311, 16320, 16519	K	K1a1
101	NAF	FL	73, 263, 315+C, 497, 750, 1189, 1438, 1811, 2706, 3480, 4769, 5583, 7028, 8224, 8860, 9055, 9698, 10398, 10550, 11299, 11467, 11470, 11719, 11914, 12007, 12308, 12372, 12555, 14167, 14766, 14798, 15326, 15412, 15924, 16224, 16311, 16519	K	K1a1b1g
102	NAF	FL	73, 263, 315+C, 497, 750, 1189, 1438, 1811, 2706, 3480, 4769, 5583, 7028, 8224, 8860, 9055, 9698, 10398, 10550, 11299, 11467, 11470, 11719, 11914, 12007, 12308, 12372, 12555, 14167, 14766, 14798, 15326, 15412, 15924, 16224, 16311, 16519	K	K1a1b1g
109	NAF	FL	64, 93, 152, 185, 189, 236, 247, 263, 315+C, 523-524d, 750, 769, 825A, 1018, 1048, 1438, 2245, 2706, 2758, 2885, 3516A, 3594, 3866, 4104, 4312, 4586, 4769, 5096, 5231, 5442, 5460, 5603, 6185, 7028, 7256, 7521, 8428, 8468, 8566, 8655, 8701, 8860, 9042, 9305C, 9347, 9540, 9755, 9818, 10398, 10589, 10664, 10688, 10810, 10873, 10915, 11167, 11176, 11641, 11719, 11914, 12007, 12705, 12720, 13105, 13276, 13506, 13650, 13708, 13988G, 13993+G, 14308, 14766, 15136, 15326, 15431, 16148, 16168, 16172, 16187, 16223, 16230, 16311, 16320, 16519	L	L0a1a

111	NAF	FL	73, 143, 146, 152, 195, 263, 309+C, 315+C, 750, 769, 1018, 1438, 2416, 2706, 2789, 3594, 4104, 4769, 6899, 7028, 7175, 7256, 7274, 7521, 7771, 8206, 8701, 8860, 9221, 9540, 10115, 10398, 10873, 11719, 11914, 11944, 12693, 12705, 13590, 13650, 13803, 13884, 14566, 14766, 15301, 15326, 15784, 16189, 16192, 16223, 16278, 16294, 16309, 16390	L	L2a1m
113	NAF	FL	73, 263, 309+C, 315+C, 523-524d, 750, 1438, 1710C, 2706, 3450, 4769, 5773, 6221, 7028, 8701, 8860, 9449, 9540, 9605, 10086, 10310, 10373, 10398, 10873, 11002, 11719, 12705, 13105, 13914A, 14094, 14766, 15301, 15311, 15326, 15670, 15824, 15944d, 16124, 16223, 16278, 16362, 16519, 16549+C	L	L3b1a4
114	NAF	FL	73, 263, 315+C, 523-524d, 750, 1438, 2332, 2404, 2706, 3450, 4769, 5773, 6221, 7028, 8701, 8860, 9449, 9540, 10086, 10373, 10398, 10873, 11002, 11719, 12372, 12705, 13105, 13914A, 13988G, 13993+G, 14766, 15301, 15311, 15326, 15824, 15944d, 16145, 16223, 16278, 16362, 16519	L	L3b1a8
107	NAF	FL	73, 195, 263, 309+C, 315+C, 489, 750, 1438, 1842, 2706, 4769, 5250, 5351, 5484, 6446, 6680, 7028, 8701, 8860, 9540, 10398, 10400, 10873, 10895, 11719, 12403, 12705, 12950C, 13111, 14110, 14766, 14783, 15043, 15301, 15326, 16129, 16183C, 16189, 16223, 16249, 16311, 16399, 16519	M	M1b2
79	NAF	FL	58, 60+T, 64, 263, 309+C, 315+C, 629, 750, 1438, 2355, 2442, 2706, 3847, 4769, 7028, 7789, 7963, 8860, 10398, 11251, 11719, 12612, 13392, 13708, 13988G, 13993+G, 14766, 15326, 15674, 16092, 16126, 16224, 16362	R0	R0a2
94	NAF	FL	73, 150, 263, 315+C, 750, 1438, 1721, 2706, 3197, 4769, 7028, 7768, 8860, 9477, 11467, 11653, 11719, 12308, 12372, 12634, 13161, 13617, 13630, 13637, 13980, 14182, 14766, 15226, 15326, 15538, 16270, 16296	U	U5b2b
97	NAF	FL	73, 185, 263, 309+C, 315+C, 523-524d, 750, 1438, 2706, 3337, 3348, 4021, 4769, 7028, 7805, 8705, 8860, 11467, 11719, 12097, 12308, 12372, 13569, 13993+G, 14179, 14766, 15326, 15790, 16172, 16183C, 16189, 16219, 16278, 16362, 16399, 16519	U	U6a3e
185	WAR	FL	263, 315+C, 750, 1438, 4769, 8860, 11914, 15326, 16519	R0	H
202	WAR	FL	152, 200, 263, 309+C, 315+C, 750, 1438, 4769, 5051, 5252, 8860, 9123T, 12941, 15326, 16519	R0	H
203	WAR	FL	152, 200, 263, 309+C, 315+C, 750, 1438, 4769, 5051, 5252, 8860, 9123T, 12941, 15326, 16519	R0	H
205	WAR	FL	263, 309+C, 315+C, 750, 1438, 4769, 8860, 11914, 13993+G, 15326, 16519	R0	H
186	WAR	FL	263, 264, 315+C, 750, 1438, 3010, 4769, 8860, 15326, 16189, 16519	R0	H1
204	WAR	FL	263, 309+C, 315+C, 750, 1438, 4769, 7645, 8860, 10217, 13993+G, 15326, 16256, 16352	R0	H14a
187	WAR	FL	150, 152+C, 191+A, 263, 309+C, 315+C, 750, 1438, 3010, 4769, 5780, 8410, 8860, 11839, 12016A, 13143A, 14386, 15326, 16189, 16311, 16519	R0	H1ap1
364	WAR	FL	150, 152+C, 191+A, 263, 309+C, 315+C, 750, 1438, 3010, 4769, 5780, 8410, 8860, 11839, 12016A, 13143A, 14386, 15326, 16189, 16311, 16519	R0	H1ap1
188	WAR	FL	65A, 150, 204, 263, 315+C, 524+AC, 750, 1438, 3010, 4769, 7762, 8860, 13993+G, 15326, 16223, 16519	R0	H1av1
189	WAR	FL	65A, 150, 204, 263, 315+C, 524+AC, 750, 1438, 3010, 4769, 7762, 8860, 13993+G, 15326, 16223, 16519	R0	H1av1
190	WAR	FL	65A, 150, 204, 263, 315+C, 524+AC, 750, 1438, 3010, 4769, 7762, 8860, 13993+G, 15326, 16223, 16519	R0	H1av1
191	WAR	FL	65A, 150, 204, 263, 315+C, 524+AC, 750, 1438, 3010, 4769, 7762, 8860, 13708, 13988G, 13993+G, 15326, 16519	R0	H1av1
363	WAR	FL	65A, 150, 204, 263, 315+C, 524+AC, 750, 1438, 3010, 4769, 7762, 8860, 13993+G, 15326, 16223, 16519	R0	H1av1
196	WAR	FL	93, 152, 263, 309+CC, 315+C, 750, 1438, 3203, 4769, 6776, 8860, 11839, 12016A, 13143A, 15326, 16111, 16239G	R0	H3a
197	WAR	FL	73, 263, 309+C, 315+C, 571, 750, 761, 1438, 4769, 6776, 8860, 13143A, 13708, 13988G, 13993+G, 15326, 15777T, 16519	R0	H3d
198	WAR	FL	73, 263, 309+C, 315+C, 571, 750, 761, 1438, 4769, 6776, 8860, 13708, 13988G, 13993+G, 15326, 15777T, 16519	R0	H3d
199	WAR	FL	263, 315+C, 750, 961, 965+XC, 1438, 1888, 4769, 6776, 8860, 15326, 16519	R0	H3r1
200	WAR	FL	146, 263, 309+C, 315+C, 750, 1438, 3613, 4769, 5999, 6776, 8860, 11839, 12016A, 15326, 16248	R0	H3w
206	WAR	FL	263, 315+C, 750, 1438, 4767, 4769, 8860, 9495, 9497, 13993+G, 15326, 16519	R0	H92
207	WAR	FL	263, 315+C, 750, 1438, 4767, 4769, 8860, 9495, 9497, 15326, 16519	R0	H92
173	WAR	FL	199, 263, 315+C, 750, 1438, 2706, 4769, 6221, 6371, 7028, 7258, 8860, 11719, 12705, 14323, 15326, 16311, 16391	R0	HV

174	WAR	FL	263, 315+C, 750, 1438, 2706, 4769, 6221, 6371, 7028, 7258, 8860, 11719, 12705, 14323, 15326, 16311, 16519	R0	HV
168	WAR	FL	263, 309+C, 315+C, 750, 1438, 2706, 3801, 4596, 4769, 7028, 8014T, 8277, 8278+XC, 8279, 8860, 14022, 15218, 15326, 16067, 16343, 16519	R0	HV1a2
169	WAR	FL	152, 263, 315+C, 750, 1438, 2706, 4769, 7028, 7094, 7805, 8763, 8860, 14515, 15326, 16129	R0	HV4a2
170	WAR	FL	152, 263, 750, 1438, 2706, 4769, 7028, 7094, 7805, 8763, 8860, 14515, 15326, 16129	R0	HV4a2
171	WAR	FL	152, 263, 750, 1438, 2706, 4769, 7028, 7094, 7805, 8763, 8860, 14515, 15326, 16129	R0	HV4a2
157	WAR	FL	73, 150, 189, 193, 200, 263, 295, 309+C, 315+C, 462, 489, 750, 1438, 2355, 2442, 2706, 3010, 3847, 4216, 4769, 5981, 6366, 7028, 8860, 9337, 13188, 13708, 13988G, 13993+G, 14152, 14212, 14766, 15301, 15326, 15452, 15670, 15674, 15942, 16069, 16126, 16193, 16223, 16263, 16300, 16309, 16327	J	J1
367	WAR	FL	73, 150, 189, 193, 200, 263, 295, 309+C, 315+C, 489, 750, 1438, 1733, 2352, 2706, 3010, 4216, 4769, 5182, 5267, 6221, 7028, 8269, 8860, 10398, 11251, 11719, 12612, 13708, 13988G, 13993+G, 14152, 14212, 14766, 15301, 15326, 15452A, 15670, 15942, 16069, 16126, 16145, 16222, 16223, 16261, 16327	J	J1b2
222	WAR	FL	73, 150, 263, 315+C, 497, 750, 1127M, 1393, 1438, 1811, 2706, 3308, 3480, 3849, 4769, 7028, 8860, 9055, 9698, 10398, 10550, 11299, 11467, 11719, 11722, 12308, 12372, 14167, 14766, 14798, 15326, 16182C, 16183C, 16189, 16224, 16311, 16519	K	K
223	WAR	FL	73, 150, 189, 263, 309+C, 315+C, 497, 573+XC, 750, 1189, 1393, 1438, 1811, 2706, 3480, 4769, 5581, 7028, 7897, 8860, 9055, 9698, 10398, 10550, 11299, 11467, 11719, 12308, 12372, 12523, 14167, 14766, 14798, 15326, 15398, 16093, 16224, 16311	K	K
224	WAR	FL	73, 150, 189, 263, 309+C, 315+C, 497, 573+XC, 750, 1189, 1393, 1438, 1811, 2706, 3480, 4769, 5581, 7028, 7897, 8860, 9055, 9698, 10398, 10550, 11299, 11467, 11719, 12308, 12372, 12523, 14167, 14766, 14798, 15326, 15398, 16093, 16224, 16311	K	K
369	WAR	FL	73, 150, 189, 263, 309+C, 315+C, 497, 573+XC, 750, 1189, 1393, 1438, 1811, 2706, 3480, 4769, 5581, 7028, 7897, 8860, 9055, 9698, 10398, 10550, 11299, 11467, 11719, 12308, 12372, 12523, 14167, 14766, 14798, 15326, 15398, 16093, 16224, 16311	K	K1a
233	WAR	FL	93, 146, 152, 185, 189, 204, 207, 247, 263, 315+C, 511, 750, 769, 825A, 1018, 1048, 1438, 1598, 1719, 1824, 2245, 2706, 2758, 2885, 3516A, 3594, 3765C, 4104, 4117, 4194, 4312, 4562, 4586, 4769, 4964, 5004, 5442, 5603, 6185, 7028, 7146, 7148, 7256, 7521, 8468, 8655, 8701, 8860, 9042, 9347, 9540, 9581, 9620, 9670, 9818, 10398, 10532, 10589, 10664, 10688, 10790, 10810, 10873, 10915, 11200C, 11287, 11641, 11719, 11914, 12007, 12678, 12705, 12720, 13105, 13276, 13470, 13650, 13680, 13899, 13928C, 14109, 14305, 14620, 14766, 14978, 15136, 15326, 15431, 15852, 16129, 16169, 16172, 16187, 16189, 16223, 16230, 16278, 16311, 16327, 16368	L	L0f2a
234	WAR	FL	152, 185, 189, 247, 263, 315+C, 511, 750, 769, 825A, 1018, 1438, 1598, 1719, 1824, 2245, 2706, 2758, 2885, 3516A, 3594, 4104, 4117, 4194, 4312, 4562, 4586, 4769, 4964, 5004, 5442, 5603, 6185, 7028, 7146, 7148, 7256, 7521, 8468, 8655, 8701, 8860, 9042, 9347, 9540, 9581, 9620, 9670, 9818, 10398, 10532, 10589, 10664, 10688, 10790, 10810, 10873, 10915, 11200C, 11287, 11641, 11719, 11914, 12007, 12678, 12684, 12705, 12720, 13105, 13276, 13470, 13650, 13680, 13928C, 14109, 14305, 14620, 14766, 14978, 15136, 15326, 15431, 15852, 16129, 16169, 16172, 16187, 16189, 16209, 16223, 16230, 16278, 16311, 16327, 16368, 16519	L	L0f2a
235	WAR	FL	152, 185, 189, 247, 263, 315+C, 511, 750, 769, 825A, 1018, 1438, 1598, 1719, 1824, 2245, 2706, 2758, 2885, 3516A, 3594, 4104, 4117, 4194, 4312, 4562, 4586, 4769, 4964, 5004, 5442, 5603, 6185, 7028, 7146, 7148, 7256, 7521, 8468, 8655, 8701, 8860, 9042, 9347, 9540, 9581, 9620, 9670, 9818, 10398, 10532, 10589, 10664, 10688, 10790, 10810, 10873, 10915, 11200C, 11287, 11641, 11719, 11914, 12007, 12678, 12684, 12705, 12720, 13105, 13276, 13470, 13650, 13680, 13928C, 14109, 14305, 14620, 14766, 14978, 15136, 15326, 15431, 15852, 16129, 16169, 16172, 16187, 16189, 16209, 16223, 16230, 16278, 16311, 16327, 16368, 16519	L	L0f2a
236	WAR	FL	73, 146, 152, 182, 185T, 195, 247, 263, 315+C, 357, 523-524d, 709, 723, 750, 769, 825A, 1018, 1738, 2153, 2352, 2706, 2758, 2768, 2885, 3308, 3594, 3666, 3693, 4104, 4619, 4769, 5036, 5046, 5393, 5655, 6548, 6827, 6989, 7028, 7055, 7146, 7256, 7389, 7521, 7674, 7867, 8248, 8277, 8468, 8655, 8701, 8860, 9540, 10398, 10688, 10810, 10873, 11719, 12519, 12705, 13105, 13506, 13650, 13789, 13880A, 13988G, 13993+G, 14178, 14203, 14560, 14766, 14769, 15115, 15326, 16126, 16172, 16187, 16189, 16223, 16264, 16270, 16278, 16293, 16311, 16360, 16519	L	L1b1a
237	WAR	FL	73, 150, 152, 182, 185T, 189, 195, 247, 263, 315+C, 357, 523-524d, 709, 750, 769, 825A, 1018, 1738, 2352, 2706, 2758, 2768, 2885, 3308, 3594, 3666, 3693, 4104, 4769, 5036, 5046, 5393, 5655, 6446, 6548, 6827, 6989, 7028, 7055, 7146, 7256, 7389, 7521, 7867, 8248, 8468, 8655, 8701, 8860, 9540, 10398, 10688, 10810, 10873, 11719, 12519, 12705, 13105, 13506, 13650, 13708, 13789, 13880A, 13988G, 13993+G, 14178, 14203, 14560, 14766, 14769, 15115, 15326, 16126, 16187, 16189, 16223, 16264, 16270, 16278, 16293, 16311, 16519	L	L1b1a9
243	WAR	FL	73, 146, 152, 189, 193, 195, 200, 263, 309+C, 315+C, 750, 769, 1018, 1438, 2358, 2416, 2706, 2789, 3594, 4104, 4317, 4769, 7028, 7175, 7256, 7274, 7521, 7771, 8206, 8701, 8860, 9221, 9540, 10115, 10143, 10398, 10873, 11656, 11719, 11914, 11944, 12693, 12705, 13590, 13650, 13708, 13803, 13988G, 13993+G, 14152, 14212, 14566, 14766, 15301, 15326, 15784, 15942, 16189, 16192, 16223, 16278, 16294, 16309, 16327, 16390	L	L2a1b
258	WAR	FL	73, 146, 189, 195, 263, 315+C, 523-524d, 750, 769, 1438, 2352, 2416, 2706, 2789, 3594, 4104, 4317, 4769, 7028, 7175, 7256, 7521, 7771, 8206, 8701, 8860, 9221, 9540, 10115, 10143, 10398, 10873, 11656, 11719, 11914, 11944, 12693, 12705, 13650, 13803, 13988G, 13993+G, 14152, 14212, 14766, 15106, 15301, 15326, 15784, 15942, 16189, 16191, 16223	L	L2a1b
244	WAR	FL	73, 143, 146, 152, 195, 263, 309+C, 315+C, 534, 750, 769, 1018, 1438, 2416, 2706, 2789, 3594, 4104, 4769, 5460, 7028, 7175, 7256, 7274, 7521, 7771, 8206, 8631, 8701, 8860, 9165, 9221, 9540, 10115, 10398, 10873, 11719, 11914, 11944, 12693, 12705, 13590, 13623, 13650, 13803, 13988G, 13993+G, 14566, 14766, 15301, 15326, 15386, 15784, 16093,	L	L2a1l

			16189, 16192, 16223, 16278, 16294, 16309, 16390		
245	WAR	FL	73, 143, 146, 150, 152, 195, 263, 315+C, 750, 769, 1018, 1438, 2416, 2706, 2789, 3594, 4104, 4317d, 4769, 5147, 7028, 7175, 7256, 7274, 7521, 7771, 8206, 8701, 8860, 9221, 9540, 10115, 10398, 10873, 11719, 11914, 11944, 12693, 12705, 13590, 13650, 13708, 13803, 13988G, 13993+G, 14566, 14766, 15301, 15326, 15784, 16129, 16189, 16192, 16223, 16278, 16290, 16294, 16309, 16327, 16390	L	L2a1n
248	WAR	FL	73, 146, 150, 152, 182, 189, 193, 195, 198, 200, 204, 207, 263, 315+C, 418, 523-524d, 750, 769, 1018, 1438, 1442, 1706, 2332, 2358, 2416, 2706, 3594, 4104, 4158, 4370, 4767, 4769, 5027, 5046, 5331A, 5814, 6026, 6713, 7028, 7256, 7521, 7624A, 8080, 8206, 8387, 8701, 8860, 9221, 9540, 10115, 10398, 10873, 11719, 11809, 11944, 12236, 12705, 12948, 13191, 13590, 13650, 13708, 13924, 13988G, 13993+G, 14059, 14766, 15110, 15217, 15301, 15326, 15460, 16114A, 16129, 16183, 16189, 16213, 16223, 16278, 16362, 16390	L	L2b1
251	WAR	FL	73, 263, 309+C, 315+C, 372, 523-524d, 750, 1438, 2706, 3450, 4769, 5773, 5853, 6221, 7028, 8347, 8701, 8860, 9449, 9540, 10086, 10373, 10398, 10873, 11002, 11719, 12705, 13105, 13988G, 13993+G, 14766, 15301, 15326, 15824, 15944d, 16124, 16223, 16278, 16519	L	L3b1a
255	WAR	FL	73, 150, 152, 189, 193, 200, 263, 315+C, 750, 1438, 2352, 2706, 4769, 6071, 7028, 7146, 7256, 7389, 7521, 8027, 8251, 8417, 8468, 8655, 8701, 8860, 9072, 9540, 10398, 10586, 10688, 10810, 10873, 11302, 11317, 11719, 11893, 12076, 12172, 12400, 12542, 12705, 12810, 13105, 13485, 13506, 13708, 13988G, 13993+G, 14212, 14766, 15301, 15326, 15670, 16223, 16327	L	L3c'd
256	WAR	FL	73, 150, 152, 189, 193, 200, 263, 315+C, 750, 1438, 2352, 2706, 4769, 6071, 7028, 7146, 7256, 7389, 7521, 8027, 8251, 8417, 8468, 8655, 8701, 8860, 9072, 9540, 10398, 10586, 10688, 10810, 10873, 11302, 11317, 11719, 11893, 12076, 12172, 12400, 12542, 12705, 12810, 13105, 13485, 13506, 13708, 13988G, 13993+G, 14212, 14766, 15301, 15326, 15670, 16223, 16327	L	L3c'd
257	WAR	FL	73, 152, 263, 315+C, 750, 921, 1438, 1719, 2706, 4769, 5147, 6722, 6935, 7028, 7389, 7424, 8618, 8701, 8860, 9540, 10398, 10550, 10873, 11719, 12046T, 12342, 12705, 13105, 13752, 13886, 14284, 14766, 15106, 15301, 15326, 16124, 16223	L	L3d1
259	WAR	FL	73, 152, 263, 315+C, 523-524d, 750, 921, 1438, 1719, 2706, 4769, 5147, 6722, 6935, 7028, 7389, 7424, 8618, 8701, 8860, 9540, 10398, 10550, 10873, 11719, 12046T, 12342, 12705, 13105, 13752, 13886, 13988G, 13993+G, 14284, 14766, 15061, 15106, 15301, 15326, 16124, 16223	L	L3d3b
210	WAR	FL	73, 150, 189, 193, 199, 263, 309+C, 315+C, 750, 1438, 2352, 2706, 4769, 6221, 7028, 7624, 8080, 8542, 8860, 9540, 9656, 10115, 10345, 10398, 10972, 11467, 11719, 11809, 12236, 12308, 12372, 12414, 13191, 13708, 13988G, 13993+G, 14152, 14212, 14766, 15301, 15326, 15545, 15553, 15670, 15942, 16189, 16223, 16327, 16343	L	L3e
219	WAR	FL	73, 143, 146, 150, 152, 189, 193, 195, 199, 263, 309+C, 315+C, 750, 769, 1018, 1438, 2352, 2416, 2594, 2706, 2789, 4769, 6221, 7028, 8860, 9055, 9698, 10398, 10490, 10550, 11299, 11467, 11719, 11992, 12308, 12338, 12372, 13590, 13708, 13988G, 13993+G, 14152, 14212, 14766, 15301, 15326, 15670, 15784, 15942, 16189, 16191, 16223, 16294, 16309, 16327, 16390	L	L3e
225	WAR	FL	73, 150, 189, 193, 200, 263, 309+C, 315+C, 750, 1438, 2352, 2706, 4769, 6221, 7028, 8860, 11719, 11794, 13708, 13988G, 13993+G, 14152, 14212, 14766, 15301, 15326, 15670, 15942, 16223, 16297A, 16327	L	L3e
262	WAR	FL	73, 152, 189, 200, 263, 309+C, 315+C, 750, 1438, 1822, 2706, 3396, 4218, 4769, 5601, 7028, 7819A, 8527, 8701, 8860, 8932, 9540, 9950, 10398, 10750, 10873, 11242, 11440, 11719, 12705, 14766, 14769, 15301, 15326, 15514, 15944d, 16209, 16223, 16292, 16311, 16519	L	L3f1b
263	WAR	FL	73, 189, 200, 263, 315+C, 750, 1438, 1822, 2706, 3396, 4218, 4769, 5601, 7028, 7235A, 7819A, 8527, 8701, 8860, 8932, 9540, 9950, 10398, 10873, 11440, 11719, 12705, 14766, 14769, 15301, 15326, 15514, 16172, 16209, 16223, 16266A, 16292, 16311, 16519	L	L3f1b2a
231	WAR	FL	73, 195, 263, 309+C, 315+C, 489, 750, 813, 1438, 1503, 2706, 4769, 5253, 6446, 6671, 6680, 7028, 8701, 8860, 9380, 9540, 10398, 10400, 10873, 11719, 12403, 12414, 12705, 12950C, 13637, 14110, 14766, 14783, 15043, 15301, 15326, 16183C, 16189, 16249, 16265C, 16311, 16519	M	M1a3b1
371	WAR	FL	65A, 150, 204, 263, 315+C, 524+AC, 750, 1438, 3010, 4769, 7762, 8860, 13993+G, 15326, 16223, 16519	M	M1a3b1
226	WAR	FL	73, 152, 204, 207, 263, 315+C, 750, 1438, 1598, 1703, 1719, 2639, 2706, 3921A, 4769, 4904, 4960, 5471, 7028, 8251, 8472, 8836, 8860, 9335, 10238, 11362, 11719, 12501, 12822, 14766, 15326, 16145, 16176G, 16223, 16362, 16390, 16519, 16535+T	N	N1b1a2
228	WAR	FL	73, 150, 189, 193, 200, 263, 309+C, 315+C, 709, 750, 1438, 2352, 2706, 4769, 5046, 6221, 7028, 8251, 8614, 8860, 8994, 11674, 11719, 11947, 12414, 12705, 13708, 13988G, 13993+G, 14152, 14212, 14766, 15301, 15326, 15670, 15884, 15942, 16223, 16265, 16292, 16325, 16327, 16519	L	N2a
154	WAR	FL	58, 60+T, 64, 263, 309+C, 315+C, 629, 750, 1438, 2355, 2442, 2706, 3847, 4769, 7028, 8860, 9337, 13188, 14766, 15326, 15674, 16092, 16126, 16224, 16362	R0	R0a2
155	WAR	FL	58, 60+T, 64, 263, 309+C, 315+C, 629, 750, 1438, 2355, 2442, 2706, 3847, 4769, 7028, 8860, 9337, 13188, 14766, 15326, 15674, 16092, 16126, 16224, 16362	R0	R0a2
156	WAR	FL	58, 60+T, 64, 150, 189, 193, 200, 263, 309+C, 315+C, 750, 1438, 2355, 2442, 2706, 3847, 4769, 5981, 6366, 7028, 8860, 13188, 13708, 13988G, 13993+G, 14766, 15326, 15674, 16126, 16304, 16362	R0	R0a2c
209	WAR	FL	73, 150, 185, 189, 193, 199, 263, 309+C, 315+C, 523-524d, 750, 1438, 2352, 2706, 3337, 3348, 4021, 4769, 6221, 7028, 7805, 8705, 8860, 11467, 11719, 12097, 12308, 12372, 13372, 13569, 13708, 13988G, 13993+G, 14152, 14179, 14212, 14766, 15301, 15326, 15670, 15790, 15942, 16172, 16219, 16223, 16278, 16327	U	U
211	WAR	FL	73, 150, 189, 193, 195, 200, 263, 285, 315+C, 385, 523d, 750, 1438, 2218, 2352, 2706, 3158, 3591, 4769, 4991, 5899, 6026, 7028, 7581, 8860, 9157, 9302, 9956, 10586, 11467,	U	U1a1a

			11719, 12308, 12372, 12879, 13104, 13422, 13708, 13988G, 13993+G, 14070, 14152, 14212G, 14364, 14766, 15148, 15301, 15326, 15670, 15942, 15954C, 16129, 16189, 16223, 16249, 16274, 16288, 16327, 16362, 16519		
212	WAR	FL	73, 146, 263, 285, 309+C, 315+C, 513, 750, 1438, 2218, 2706, 4769, 4991, 6026, 7028, 7403, 7581, 8860, 11467, 11719, 12308, 12372, 12879, 13708, 13988G, 13993+G, 14070, 14364, 14766, 15115, 15148, 15217, 15326, 15954C, 16188, 16189, 16223, 16249, 16519	U	U1a1c
208	WAR	FL	73, 150, 189, 193, 199, 263, 309+C, 315+C, 750, 1438, 2352, 2706, 4769, 6221, 7028, 8860, 9477, 10619, 11467, 11719, 12308, 12372, 13708, 13988G, 13993+G, 14152, 14212, 14766, 15301, 15326, 15670, 15942, 16223	U	U6a3e
215	WAR	FL	152, 263, 309+C, 315+C, 523-524d, 750, 980, 1438, 1811, 2706, 3741, 4769, 5360, 7028, 8137, 8684, 8860, 10084, 10142, 11467, 11719, 12308, 12372, 13500, 14569, 14766, 15326, 16184, 16318T, 16381, 16519	U	U7b
216	WAR	FL	152, 263, 309+C, 315+C, 523-524d, 750, 980, 1438, 1811, 2706, 3741, 4769, 5360, 7028, 8137, 8684, 8860, 10084, 10142, 11467, 11719, 12308, 12372, 13500, 14569, 14766, 15326, 16184, 16318T, 16381, 16519	U	U7b
177	WAR	FL	72, 263, 309+CT, 310, 315+C, 750, 1438, 2706, 4580, 4769, 7028, 8860, 13708, 13988G, 13993+G, 14488, 14629, 14861, 15326, 15904, 16298	R0	V
178	WAR	FL	72, 263, 309+CT, 310, 315+C, 750, 1438, 2706, 4580, 4769, 7028, 8860, 13708, 13988G, 13993+G, 14488, 14629, 14861, 15326, 15904, 16298	R0	V
230	WAR	FL	73, 153, 195, 225, 226, 249d, 260, 263, 309+CT, 310, 315+C, 750, 1438, 1719, 2706, 4769, 6221, 6371, 7028, 8393, 8860, 10388, 11719, 12705, 13708, 13966, 14470, 14766, 15148, 15326, 15927, 16183C, 16189, 16223, 16278, 16519	X	X2b
39	ZOU	FL	146, 263, 315+C, 750, 1438, 4769, 6261, 8860, 13993+G, 15326, 15670, 16519	R0	H
45	ZOU	FL	263, 315+C, 750, 1438, 1700, 4769, 8860, 9438, 9554, 13708, 13988G, 13993+G, 15326, 16145, 16519	R0	H
46	ZOU	FL	263, 315+C, 750, 1438, 1700, 4769, 8860, 9438, 9554, 13708, 13988G, 13993+G, 15326, 16145, 16519	R0	H
40	ZOU	FL	263, 309+C, 315+C, 750, 1438, 3010, 3083, 4769, 8860, 12918, 15326, 16519	R0	H1
44	ZOU	FL	263, 8860, 10724, 11719, 12705, 15326, 16519	R0	H2a2
29	ZOU	FL	152, 263, 315+C, 593, 750, 1438, 1530, 2706, 3547, 4769, 6023, 7028, 8014T, 8860, 12070, 12696, 13708, 13993+G, 15218, 15326, 16067, 16189, 16266G, 16519	R0	HV1b2
10	ZOU	FL	73, 185, 228, 263, 295, 315+C, 462, 482, 489, 750, 1438, 2706, 3010, 3394, 4216, 4769, 5082, 7028, 7627, 8860, 10398, 11251, 11719, 12612, 13708, 13993+G, 14766, 14798, 15326, 15452A, 15580, 16069, 16126	J	J1c1
11	ZOU	FL	73, 185, 228, 263, 295, 315+C, 462, 482, 489, 750, 1438, 2706, 3010, 3394, 4216, 4769, 5082, 7028, 7627, 8860, 10398, 11251, 11719, 12612, 13708, 13988G, 13993+G, 14766, 14798, 15326, 15452A, 15580, 16069, 16126	J	J1c1
12	ZOU	FL	73, 185, 228, 263, 295, 315+C, 462, 482, 489, 750, 1438, 2706, 3010, 3394, 4216, 4769, 5082, 7028, 7627, 8860, 10398, 11251, 11719, 12612, 13708, 13988G, 13993+G, 14766, 14798, 15326, 15452A, 15580, 16069, 16126	J	J1c1
13	ZOU	FL	73, 185, 228, 263, 295, 315+C, 462, 482, 489, 750, 1438, 2706, 3010, 3394, 4216, 4769, 5082, 7028, 7627, 8860, 10398, 11251, 11719, 12612, 13708, 13993+G, 14766, 14798, 15326, 15452A, 15580, 16069, 16126	J	J1c1
14	ZOU	FL	73, 185, 228, 263, 295, 315+C, 462, 482, 489, 750, 1438, 2706, 3010, 3394, 4216, 4769, 5082, 7028, 7627, 8860, 10398, 11251, 11719, 12612, 13708, 13993+G, 14766, 14798, 15326, 15452A, 15580, 16069, 16126	J	J1c1
15	ZOU	FL	73, 185, 228, 263, 295, 315+C, 462, 482, 489, 750, 1438, 2706, 3010, 3394, 4216, 4769, 5082, 7028, 7627, 8860, 10398, 11251, 11719, 12612, 13708, 13988G, 13993+G, 14766, 14798, 15326, 15452A, 15580, 16069, 16126	J	J1c1
16	ZOU	FL	73, 185, 228, 263, 295, 315+C, 462, 482, 489, 750, 1438, 2706, 3010, 3394, 4216, 4769, 5082, 7028, 7627, 8860, 10398, 11251, 11719, 12612, 13708, 14766, 14798, 15326, 15452A, 15580, 16069, 16126	J	J1c1
17	ZOU	FL	73, 185, 228, 263, 295, 315+C, 462, 482, 489, 750, 1438, 2706, 3010, 3394, 4216, 4769, 5082, 7028, 7627, 8860, 10398, 11251, 11719, 12612, 13708, 13993+G, 14766, 14798, 15326, 15452A, 15580, 16069, 16126	J	J1c1
18	ZOU	FL	73, 185, 228, 263, 295, 315+C, 462, 482, 489, 750, 1438, 2706, 3010, 3394, 4216, 4769, 5082, 7028, 7627, 8860, 10398, 11251, 11719, 12612, 13708, 14766, 14798, 15326, 15452A, 15580, 16069, 16126	J	J1c1
19	ZOU	FL	73, 185, 228, 263, 295, 315+C, 462, 482, 489, 750, 1438, 2706, 3010, 3394, 4216, 4769, 5082, 7028, 7627, 8860, 10398, 11251, 11719, 12612, 13708, 14766, 14798, 15326, 15452A, 15580, 16069, 16126	J	J1c1
20	ZOU	FL	73, 185, 228, 263, 295, 315+C, 462, 482, 489, 750, 1438, 2706, 3010, 3394, 4216, 4769, 5082, 7028, 7627, 8860, 10398, 11251, 11719, 12612, 13708, 14766, 14798, 15326, 15452A, 15580, 16069, 16126	J	J1c1

21	ZOU	FL	73, 185, 228, 263, 295, 315+C, 462, 482, 489, 750, 1438, 2706, 3010, 3394, 4216, 4769, 5082, 7028, 7627, 8860, 10398, 11251, 11719, 12612, 13708, 13988G, 13993+G, 14766, 14798, 15326, 15452A, 15580, 16069, 16126	J	J1c1
22	ZOU	FL	73, 185, 228, 263, 295, 315+C, 462, 482, 489, 750, 1438, 2706, 3010, 3394, 4216, 4769, 5082, 7028, 7627, 8860, 10398, 11251, 11719, 12612, 13708, 14766, 14798, 15326, 15452A, 15580, 16069, 16126	J	J1c1
23	ZOU	FL	73, 185, 228, 263, 295, 315+C, 462, 482, 489, 750, 1438, 2706, 3010, 3394, 4216, 4769, 5082, 7028, 7627, 8860, 10398, 11251, 11719, 12612, 13708, 13988G, 13993+G, 14766, 14798, 15326, 15452A, 15580, 16069, 16126	J	J1c1
24	ZOU	FL	73, 185, 228, 263, 295, 315+C, 462, 482, 489, 750, 1438, 2706, 3010, 3394, 4216, 4769, 5082, 7028, 7627, 8860, 10398, 11251, 11719, 12612, 13708, 13988G, 13993+G, 14766, 14798, 15326, 15452A, 15580, 16069, 16126	J	J1c1
25	ZOU	FL	73, 185, 228, 263, 295, 315+C, 462, 482, 489, 750, 1438, 2706, 3010, 3394, 4216, 4769, 5082, 7028, 7627, 8860, 10398, 11251, 11719, 12612, 13708, 13988G, 13993+G, 14766, 14798, 15326, 15452A, 15580, 16069, 16126	J	J1c1
72	ZOU	FL	73, 152, 185T, 189, 195, 247, 263, 315+C, 357, 523-524d, 709, 750, 769, 825A, 1018, 1738, 2352, 2706, 2758, 2768, 2885, 3308, 3316C, 3594, 3666, 3693, 4104, 4769, 5036, 5046, 5393, 5655, 6548, 6827, 6989, 7028, 7055, 7146, 7256, 7270, 7389, 7521, 7867, 8248, 8468, 8655, 8701, 8860, 9540, 10398, 10688, 10810, 10873, 11719, 12519, 12609, 12705, 13105, 13506, 13650, 13708, 13789, 13988G, 13993+G, 14178, 14203, 14560, 14766, 14769, 15115, 15326, 16086, 16126, 16187, 16189, 16223, 16245, 16247, 16264, 16270, 16278, 16293, 16311, 16519	L	L1b1a18
73	ZOU	FL	73, 146, 152, 195, 263, 315+C, 750, 769, 1018, 1438, 2416, 2706, 2789, 3594, 4104, 4769, 5894C, 7028, 7175, 7256, 7274, 7521, 7702, 7771, 8206, 8496, 8701, 8860, 9221, 9540, 10115, 10143, 10398, 10873, 11719, 11914, 11944, 12693, 12705, 13590, 13650, 13803, 13988G, 13993+G, 14566, 14766, 15301, 15326, 15784, 16086, 16184, 16189, 16192, 16223, 16278, 16294, 16309, 16390	L	L2a1b
58	ZOU	FL	73, 143, 146, 150, 152, 189, 193, 195, 199, 263, 309+C, 315+C, 750, 769, 1018, 1438, 2352, 2416, 2594, 2706, 2789, 4769, 6221, 7028, 8860, 9055, 9698, 10398, 10490, 10550, 11299, 11467, 11719, 11992, 12308, 12338, 12372, 13590, 13708, 13988G, 13993+G, 14152, 14212, 14766, 15301, 15326, 15670, 15784, 15942, 16189, 16191, 16223, 16294, 16309, 16327, 16390	L	L3e
70	ZOU	FL	73, 195, 200, 263, 309+C, 315+C, 466, 489, 750, 1438, 2706, 4769, 4936, 6446, 6680, 7028, 8701, 8860, 8868, 9540, 10398, 10400, 10873, 11671, 11719, 12403, 12705, 12950C, 13111, 13988G, 13993+G, 14110, 14766, 14783, 15043, 15247G, 15301, 15326, 16129, 16185, 16189, 16223, 16249, 16311, 16519	M	M1b1a
68	ZOU	FL	73, 152, 195, 263, 309+C, 315+C, 750, 1406, 1438, 1598, 1703, 1719, 2639, 2706, 3921A, 4769, 4960, 5471, 7028, 8251, 8472, 8521, 8836, 8860, 9335, 9438, 10238, 11362, 11719, 12501, 12705, 12822, 13768, 13988G, 14766, 15326, 15805, 16145, 16176G, 16223, 16355, 16390, 16519	N	N1b1a7
26	ZOU	FL	73, 200, 263, 309+C, 315+C, 709, 750, 1438, 1888, 2483, 2706, 4216, 4769, 4917, 7028, 7853, 8697, 8860, 10463, 11251, 11447, 11719, 12633A, 13368, 13759, 13993+G, 14766, 14788, 14905, 15326, 15452A, 15607, 15928, 16126, 16163, 16186, 16189, 16291, 16294, 16519	T	T1a
27	ZOU	FL	73, 263, 309+C, 315+C, 709, 750, 1438, 1888, 2706, 3867, 4216, 4769, 4917, 5899+C, 7028, 8697, 8860, 10376, 10463, 11251, 11719, 12633A, 13368, 14766, 14905, 15326, 15452A, 15607, 15928, 16126, 16163, 16186, 16189, 16294, 16519	T	T1a6
28	ZOU	FL	73, 263, 309+C, 315+C, 709, 750, 1438, 1888, 2706, 3867, 4216, 4769, 4917, 5899+C, 7028, 8697, 8860, 10376, 10463, 11251, 11719, 12633A, 13368, 14766, 14905, 15326, 15452A, 15607, 15928, 16126, 16163, 16186, 16189, 16294, 16519	T	T1a6
47	ZOU	FL	263, 309+C, 315+C, 523-524d, 750, 1438, 2706, 4769, 7028, 7805, 8860, 11467, 11719, 12308, 12372, 12414, 13359, 15326, 15497, 16189	U	U
48	ZOU	FL	73, 263, 309+C, 315+C, 523-524d, 750, 1438, 2706, 4769, 7028, 7805, 8860, 11467, 11719, 12308, 12372, 12414, 13359, 15326, 15497, 16189	U	U
49	ZOU	FL	263, 309+C, 315+C, 523-524d, 750, 1438, 2706, 4769, 7028, 7805, 8860, 11467, 11719, 12308, 12372, 12414, 13359, 15326, 15497, 16189	U	U
50	ZOU	FL	73, 150, 263, 315+C, 750, 1438, 1721, 2706, 3197, 4769, 7028, 7768, 8860, 9477, 11467, 11653, 11719, 12308, 12372, 12634, 13161, 13617, 13630, 13637, 13980, 14182, 14766, 15226, 15326, 15538, 16270, 16296	U	U5b2b
55	ZOU	FL	73, 146, 152, 185, 188, 263, 309+C, 315+C, 750, 1211, 1438, 2706, 3348, 4769, 7028, 7805, 8860, 11268, 11467, 11719, 12308, 12372, 13431, 13708, 13988G, 13993+G, 14148, 14179, 14766, 15326, 15634, 15790, 16172, 16182C, 16183d, 16189, 16219, 16278	U	U6a3b
56	ZOU	FL	73, 185, 263, 309+C, 315+C, 523-524d, 750, 1438, 2706, 3337, 3348, 4021, 4769, 7028, 7805, 8705, 8860, 11467, 11719, 12097, 12308, 12372, 13569, 13928, 13993+G, 14179, 14766, 15326, 15790, 16172, 16183C, 16189, 16219, 16278, 16362, 16399	U	U6a3e
57	ZOU	FL	73, 103, 143, 263, 309+C, 315+C, 1438, 2706, 2775, 3348, 4769, 5813, 7028, 7805, 7813, 8282, 8860, 9752, 10172, 11467, 11539, 11719, 12308, 12372, 13781, 13988G, 13993+G, 14179, 14766, 15326, 16172, 16189, 16219, 16278	U	U6a8a
69	ZOU	FL	73, 146, 153, 263, 309+CCT, 310, 315+C, 750, 1438, 2706, 4769, 5302, 6221, 6371, 6713, 7028, 7337, 8860, 9615, 10640, 11719, 12705, 13966, 14470, 14587, 14766, 15326, 15654, 16104, 16181C, 16182C, 16183d, 16189, 16519	X	X1c

Table 19. Y-chromosome haplotypes in Libya

(AFL, African origin; EAR, Eastern Libyan Arab; WAL, Western Libyan Arab; NAS, Nafusa Berber; Zou, Zuwara Berber; Gha, Ghadames Berber)

		DYS 393	DYS 390	DYS 19	DYS 391	DYS 385a	DYS 385b	DYS 439	DYS389I	DYS 392	DYS 389II	DYS 458	DYS 437	DYS 448	H4	DYS 576	DYS 570	DYS 438	DYS 481	DYS 549	DYS 533	DYS 635	DYS 643	Haplogroup
LB10	West	13	24	13	11	19	21	13	14	11	31	14	14	19	12	19	19	11	22	12	12	22	11	E-M78 E1b1b V13
LB2	East	13	23	13	11	17	17	13	13	11	32	16	14	20	11	18	18	10	23	12	11	24	12	E-M78 E1b1b V13
LB3	Gha	13	24	13	11	17	18	12	13	11	31	19	14	21	12	19	18	11	25	12	11	22	12	E-M78 E1b1b V13
LB4	East	13	23	13	11	17	17	13	13	11	32	16	14	20	11	18	18	10	23	12	11	24	12	E-M78 E1b1b V13
LB5	Gha	13	24	13	11	17	18	12	13	11	31	19	14	21	12	19	18	11	25	12	11	22	12	E-M78 E1b1b V13
LB6	West	13	23	14	11	17	19	11	12	11	29	14	14	20	11	18	19	11	23	12	12	21	11	E-M78 E1b1b V13
LB7	West	13	23	13	11	18	18	11	12	11	29	14	14	20	11	18	19	11	21	13	11	22	12	E-M78 E1b1b V13
LB8	West	13	23	13	11	18	18	11	13	11	29	14	14	20	11	18	19	11	21	13	11	22	12	E-M78 E1b1b V13
LB9	West	13	24	13	11	19	21	13	14	11	31	14	14	19	12	19	19	11	22	12	12	22	11	E-M78 E1b1b V13
LB1	East	13	23	13	11	17	17	13	13	11	32	16	14	20	11	18	18	10	23	12	11	24	12	E-M78 E1b1b V12
LB11	West	13	24	11	11	18	18	11	13	12	30	13	14	20	12	18	18	13	24	13	13	20	14	E-M78 E1b1b V12
LB12	West	13	24	15	11	18	22	11	13	12.2	30	13	14	20	12	18	18	12	24	13	13	20	15	E-M78 E1b1b V12
LB13	West	13	24	11	11	18	18	11	13	12	30	13	14	20	12	18	18	13	24	13	13	20	14	E-M78 E1b1b V12
LB14	West	13	23	15	11	18	22	11	13	12.2	30	13	14	20	12	18	18	12	24	13	13	20	15	E-M78 E1b1b V12
LB15	Gha	13	24	13	11	19	20	12	14	11	31	15	14	20	13	19	19	11	22	13	12	21	12	E-M78 E1b1b V22
LB16	Gha	13	24	13	11	18	20	12	14	12	31	15	14	20	13	19	18	11	22	13	12	21	12	E-M78 E1b1b V22

LB17	West	13	22	13	11	18	20	10	12	11	29	14	14	20	11	18	19	11	21	13	11	21	12	E-M78 E1b1b V22
LB18	West	13	24	13	11	19	20	12	14	11	31	15	14	20	13	19	19	14	22	13	11	21	12	E-M78 E1b1b V22
LB19	West	13	22	13	11	18	20	10	12	11	29	14	14	20	11	18	19	11	21	13	11	21	12	E-M78 E1b1b V22
LB20	West	13	22	13	11	18	20	10	12	11	31	14	14	20	11	18	19	11	21	13	11	21	12	E-M78 E1b1b V22
LB21	West	13	24	13	11	19	20	12	14	11	31	15	14	20	13	19	19	14	22	13	11	21	12	E-M78 E1b1b V22
LB22	West	13	22	13	11	18	20	10	12	11	29	14	14	20	11	18	19	11	21	13	11	21	12	E-M78 E1b1b V22
LB23	West	13	24	13	11	19	20	12	14	11	31	15	14	20	13	19	19	14	22	13	11	21	12	E-M78 E1b1b V22
LB24	Zou	13	23	14	11	17	20	13	14	11	31	17	14	20	13	17	22	11	23	15	12	20	10	E-M78 E1b1b V22
LB25	Gha	13	24	13	11	19	20	12	14	11	31	15	14	20	13	19	19	11	22	13	12	21	12	E-M78 E1b1b V22
LB26	Zou	13	24	14	11	17	20	13	14	11	31	15	14	20	13	19	19	11	23	13	12	21	10	E-M78 E1b1b V22
LB27	Zou	13	24	10	11	17.2	20.2	12	14	11.2	31	18.2	14	20	13	19	19	10	22	13	12	21	13	E-M78 E1b1b V22
LB28	Naf	13	24	14	10	14	16	10	13	11	31	18	14	21	12	17	21	10	24	11	11	21	10.1	E1b1b M123 M34 M84
LB29	Naf	13	24	14	10	14	16	10	13	11	31	18	14	21	12	17	21	10	24	11	11	21	10.1	E1b1b M123 M34 M84
LB30	Naf	13	24	14	10	14	16	10	13	11	31	18	14	21	12	17	21	10	24	11	11	21	10.1	E1b1b M123 M34 M84
LB31	Naf	13	24	14	11	14	16	10	13	11	29	18	14	21	12	18	21	10	24	11	11	21	10.1	E1b1b M123 M34 M84
LB32	Naf	13	24	14	10	14	16	10	13	11	31	18	14	21	12	17	21	10	24	11	11	21	10.1	E1b1b M123 M34 M84
LB33	Naf	13	24	14	10	14	16	10	13	11	31	18	14	21	12	17	21	10	24	11	11	21	10.1	E1b1b M123 M34 M84
LB100	Zou	13	24	13	10	15	23	10	14	11	31	19	14	20	12	18	22	11	26	10	11	21	12	E1b1b V257 M81

LB101	Zou	13	24	13	10	14	15	10	14	11	30	21	14	21	12	18	22	11	26	11	11	21	10	E1b1b V257 M81
LB102	Zou	13	24	14	10	14	15	10	14	11	31	18	14	21	12	18	21	11	25	11	11	21	12	E1b1b V257 M81
LB103	Zou	13	24	13	10	13	16	10	14	11	30	18	14	20	12	18	22	11	27	11	11	21	12	E1b1b V257 M81
LB104	Zou	13	24	13	10	16	14	10	13	11	29	17	14	20	13	18	22	11	27	11	11	21	12	E1b1b V257 M81
LB105	Zou	13	23	13	10	15	15	10	14	11	29	18	14	20	12	19	22	11	28	11	11	21	12	E1b1b V257 M81
LB106	Zou	13	24	13	10	14	14	10	14	11	30	18	14	20	12	18	22	11	29	12	11	21	12	E1b1b V257 M81
LB107	Zou	13	24	13.2	10	14.2	15.2	10	14	11.2	31	17	14	17	12	19	21	10	24	11	11	22	13	E1b1b V257 M81
LB108	Zou	13	24	12	10	14.2	16.2	10	14	13	30	17	14	20	12	19	22	10	26	11	11	21	13	E1b1b V257 M81
LB109	Zou	13	21	13	10	14.2	23	10	14	11	31	18	14	20	11	19	22	10	27	11	11	21	13	E1b1b V257 M81
LB110	Zou	13	25	12	10	13.2	15.2	10	14	10.2	31	14.2	14	20	12	20	23	10	27	11	11	21	13	E1b1b V257 M81
LB111	Zou	13	24	13.2	10	14.2	15.2	10	14	11.2	31	17	14	17	11	18	21	10	25	11	11	21	13	E1b1b V257 M81
LB112	Zou	13	24	13.2	10	15.2	14.2	10	14	11.2	31	17	14	17	12	20	21	10	24	11	11	22	13	E1b1b V257 M81
LB113	Zou	15	21	15	11	15	17	15	13	15	29	15	14	20	11	17	18	12	22	11	11	21	12	E1b1b V257 M81
LB114	Zou	13	24	13	10	14	15	10	14	11	30	20	14	20	11	18	22	11	26	11	11	21	12	E1b1b V257 M81

LB115	Zou	13	24	14	10	14	15	10	14	11	31	17	14	21	11	18	21	11	25	11	11	21	12	E1b1b V257 M81
LB116	Zou	13	24	13	10	14	15	10	14	11	29	18	14	20	12	18	21	11	25	11	11	21	17	E1b1b V257 M81
LB117	Zou	12	25	13	10	13	15	10	14	11	31	15	14	20	12	20	23	11	27	11	11	21	12	E1b1b V257 M81
LB118	Zou	13	24	14	10	14	15	10	14	11	31	19	14	21	12	18	22	11	25	11	11	22	12	E1b1b V257 M81
LB119	Zou	13	24	13	10	15	23	10	14	11	31	19	14	20	12	18	22	11	26	10	11	21	12	E1b1b V257 M81
LB120	Zou	13	24	13	10	14	15	10	14	11	30	21	14	21	12	18	22	11	26	11	11	21	10	E1b1b V257 M81
LB121	Zou	13	24	14	10	14	15	10	14	11	31	18	14	21	12	18	21	11	25	11	11	21	12	E1b1b V257 M81
LB122	Zou	13	24	13	10	13	16	10	14	11	30	18	14	20	12	18	22	11	27	11	11	21	12	E1b1b V257 M81
LB123	Zou	13	24	13	10	16	14	10	13	11	29	17	14	20	13	18	22	11	27	11	11	21	12	E1b1b V257 M81
LB124	Zou	13	23	13	10	15	15	10	14	11	29	18	14	20	12	19	22	11	28	11	11	21	12	E1b1b V257 M81
LB125	Zou	13	24	13	10	14	14	10	14	11	30	18	14	20	12	18	22	11	29	12	11	21	12	E1b1b V257 M81
LB126	Zou	13	24	13.2	10	14.2	15.2	10	14	11.2	31	17	14	17	12	19	21	10	24	11	11	22	13	E1b1b V257 M81
LB127	Zou	13	24	12	10	14.2	16.2	10	14	13	30	17	14	20	12	19	22	10	26	11	11	21	13	E1b1b V257 M81
LB128	Zou	13	21	13	10	14.2	23	10	14	11	31	18	14	20	11	19	22	10	27	11	11	21	13	E1b1b V257 M81

LB129	Zou	13	25	12	10	13.2	15.2	10	14	10.2	31	14.2	14	20	12	20	23	10	27	11	11	21	13	E1b1b V257 M81
LB130	Zou	13	24	13.2	10	14.2	15.2	10	14	11.2	31	17	14	17	11	18	21	10	25	11	11	21	13	E1b1b V257 M81
LB131	Zou	13	24	13.2	10	15.2	14.2	10	14	11.2	31	17	14	17	12	20	21	10	24	11	11	22	13	E1b1b V257 M81
LB34	Gha	14	21	14.2	11	15.2	17.2	12	12	11	29	16	14	22	11	16	18	11	25	11	11	21	13	E1b1b V257 M81
LB35	Gha	13	21	15	11	15	18	21	14	11	31	18	14	21	11	13	18	12	27	12	11	21	14	E1b1b V257 M81
LB36	Gha	13	24	13	10	14	15	10	14	11	30	20	14	20	11	18	22	10	26	11	11	21	10.1	E1b1b V257 M81
LB37	Gha	13	24	13	10	14	15	10	14	11	30	18	14	20	12	18	23	10	26	11	11	21	10.1	E1b1b V257 M81
LB38	Gha	13	24	13	10	14	15	10	14	11	30	20	14	21	12	18	23	10	26	11	11	21	10.1	E1b1b V257 M81
LB39	Gha	13	24	13	10	14	15	10	14	11	30	18	14	20	12	19	22	10	26	11	11	21	10.1	E1b1b V257 M81
LB40	Gha	13	24	13	10	14	15	10	14	11	30	20	14	20	11	18	22	10	26	11	11	21	10.1	E1b1b V257 M81
LB41	Gha	13	24	13	10	14	15	10	13	11	29	18	14	20	12	18	23	10	27	11	11	21	10.1	E1b1b V257 M81
LB42	Gha	13	24	13	10	14	15	10	14	11	30	17	14	20	11	18	22	11	25	11	11	21	12	E1b1b V257 M81
LB43	Gha	13	24	13	10	14	16	10	14	11	30	16	14	21	12	18	22	11	25	11	12	21	10.1	E1b1b V257 M81
LB44	Gha	13	24	13.2	10	14	15	10	13	11	29	17	14	19	11	18	22	11	25	12	11	21	12	E1b1b V257 M81
LB45	Gha	13	24	13	10	14	15	10	13	11	29	18	14	20	12	17	22	11	27	11	11	21	12	E1b1b V257 M81
LB46	Gha	13	24	13	10	14	16	10	14	11	30	17	14	20	12	19	22	11	28	11	11	21	10.2	E1b1b V257 M81
LB47	Gha	13	25	13	10	14	16	10	13	11	30	17	14	20	12	17	22	11	29	11	11	21	12	E1b1b V257 M81
LB48	Gha	13	24	13	10	14	15	10	14	11	30	17	14	20	11	16	22	11	28	11	11	21	12	E1b1b V257 M81
LB49	Gha	14	24	14	10	16	14	10	13	10	29	18	14	21	12	19	22	8.2	27	11	11	21	12	E1b1b V257 M81
LB50	Gha	13	24	13	10	13	16	10	14	11	30	18	14	20	11	17	22	11	27	11	12	21	13	E1b1b V257 M81

LB51	**Naf**	**13**	**25**	**16**	**12**	**15**	**16**	**13**	**14**	**13**	**30**	**17**	**14**	**19**	**13**	**18**	**18**	**12**	**21**	**12**	**12**	**23**	**12**	**E1b1b V257 M81**
LB52	**West**	**14**	**21**	**15**	**11**	**18**	**18**	**12**	**14**	**11**	**31**	**18**	**14**	**20**	**11**	**15**	**19**	**12**	**21**	**11**	**12**	**22**	**13**	**E1b1b V257 M81**
LB53	**West**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**13**	**11**	**29**	**18**	**14**	**20**	**12**	**18**	**23**	**10**	**27**	**11**	**11**	**21**	**10.1**	**E1b1b V257 M81**
LB54	**West**	**13**	**24**	**13**	**11**	**16**	**15**	**10**	**13**	**11**	**29**	**17**	**14**	**20**	**12**	**18**	**22**	**11**	**26**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB55	**West**	**13**	**24**	**13**	**10**	**14**	**16**	**10**	**14**	**11**	**30**	**17**	**14**	**20**	**12**	**18**	**22**	**11**	**27**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB56	**West**	**13**	**24**	**13**	**10**	**14**	**16**	**10**	**13**	**11**	**29**	**17**	**14**	**20**	**12**	**18**	**22**	**11**	**27**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB57	**West**	**13**	**25**	**13**	**10**	**14**	**16**	**10**	**13**	**11**	**29**	**17**	**14**	**20**	**12**	**18**	**22**	**11**	**26**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB58	**West**	**13**	**24**	**13**	**10**	**14**	**16**	**10**	**13**	**11**	**29**	**17**	**14**	**20**	**12**	**18**	**22**	**11**	**25**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB59	**West**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**14**	**10**	**30**	**18**	**14**	**20**	**12**	**18**	**22**	**11**	**27**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB60	**West**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**13**	**10**	**30**	**18**	**14**	**20**	**12**	**15**	**22**	**11**	**26**	**11**	**11**	**21**	**13**	**E1b1b V257 M81**
LB61	**West**	**13**	**24**	**13**	**10**	**14**	**15**	**11**	**14**	**11**	**30**	**18**	**14**	**20**	**12**	**19**	**20**	**11**	**23**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB62	**West**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**14**	**11**	**30**	**19**	**14**	**19**	**12**	**19**	**23**	**11**	**27**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB63	**West**	**13**	**24**	**14**	**10**	**14**	**16**	**10**	**14**	**11**	**30**	**17**	**14**	**20**	**12**	**18**	**22**	**11**	**28**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB64	**West**	**13**	**24**	**13**	**10**	**14**	**16**	**10**	**13**	**11**	**29**	**17**	**14**	**20**	**12**	**19**	**22**	**11**	**27**	**11**	**11**	**22**	**12**	**E1b1b V257 M81**
LB65	**Gha**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**14**	**11**	**30**	**20**	**14**	**20**	**11**	**18**	**22**	**10**	**26**	**11**	**11**	**21**	**10.1**	**E1b1b V257 M81**
LB66	**Gha**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**14**	**11**	**30**	**18**	**14**	**20**	**12**	**18**	**23**	**10**	**26**	**11**	**11**	**21**	**10.1**	**E1b1b V257 M81**
LB67	**Gha**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**14**	**11**	**30**	**20**	**14**	**21**	**12**	**18**	**23**	**10**	**26**	**11**	**11**	**21**	**10.1**	**E1b1b V257 M81**
LB68	**Gha**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**14**	**11**	**30**	**18**	**14**	**20**	**12**	**19**	**22**	**10**	**26**	**11**	**11**	**21**	**10.1**	**E1b1b V257 M81**
LB69	**Gha**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**14**	**11**	**30**	**20**	**14**	**20**	**11**	**18**	**22**	**10**	**26**	**11**	**11**	**21**	**10.1**	**E1b1b V257 M81**
LB70	**Gha**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**13**	**11**	**29**	**18**	**14**	**20**	**12**	**18**	**23**	**10**	**27**	**11**	**11**	**21**	**10.1**	**E1b1b V257 M81**
LB71	**Gha**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**14**	**11**	**30**	**17**	**14**	**20**	**11**	**18**	**22**	**11**	**25**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**

LB72	**Gha**	**13**	**24**	**13**	**10**	**14**	**16**	**10**	**14**	**11**	**30**	**16**	**14**	**21**	**12**	**18**	**22**	**11**	**25**	**11**	**12**	**21**	**10.1**	**E1b1b V257 M81**
LB73	**Gha**	**13**	**24**	**13.2**	**10**	**14**	**15**	**10**	**13**	**11**	**29**	**17**	**14**	**19**	**11**	**18**	**22**	**11**	**25**	**12**	**11**	**21**	**12**	**E1b1b V257 M81**
LB74	**Gha**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**13**	**11**	**29**	**18**	**14**	**20**	**12**	**17**	**22**	**11**	**27**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB75	**Gha**	**13**	**25**	**13**	**10**	**14**	**16**	**10**	**13**	**11**	**30**	**17**	**14**	**20**	**12**	**17**	**22**	**11**	**29**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB76	**Gha**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**14**	**11**	**30**	**17**	**14**	**20**	**11**	**16**	**22**	**11**	**28**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB77	**Gha**	**14**	**24**	**14**	**10**	**16**	**14**	**10**	**13**	**10**	**29**	**18**	**14**	**21**	**12**	**19**	**22**	**8.2**	**27**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB78	**Gha**	**13**	**24**	**13**	**10**	**13**	**16**	**10**	**14**	**11**	**30**	**18**	**14**	**20**	**11**	**17**	**22**	**11**	**27**	**11**	**12**	**21**	**13**	**E1b1b V257 M81**
LB79	**Naf**	**13**	**25**	**16**	**12**	**15**	**16**	**13**	**14**	**13**	**30**	**17**	**14**	**19**	**13**	**18**	**18**	**12**	**21**	**12**	**12**	**23**	**12**	**E1b1b V257 M81**
LB80	**West**	**14**	**21**	**15**	**11**	**18**	**18**	**12**	**14**	**11**	**31**	**18**	**14**	**20**	**11**	**15**	**19**	**12**	**21**	**11**	**12**	**22**	**13**	**E1b1b V257 M81**
LB81	**West**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**13**	**11**	**29**	**18**	**14**	**20**	**12**	**18**	**23**	**10**	**27**	**11**	**11**	**21**	**10.1**	**E1b1b V257 M81**
LB82	**West**	**13**	**24**	**13**	**11**	**16**	**15**	**10**	**13**	**11**	**29**	**17**	**14**	**20**	**12**	**18**	**22**	**11**	**26**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB83	**West**	**13**	**24**	**13**	**10**	**14**	**16**	**10**	**14**	**11**	**30**	**17**	**14**	**20**	**12**	**18**	**22**	**11**	**27**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB84	**West**	**13**	**24**	**13**	**10**	**14**	**16**	**10**	**13**	**11**	**29**	**17**	**14**	**20**	**12**	**18**	**22**	**11**	**27**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB85	**West**	**13**	**25**	**13**	**10**	**14**	**16**	**10**	**13**	**11**	**29**	**17**	**14**	**20**	**12**	**18**	**22**	**11**	**26**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB86	**West**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**14**	**10**	**30**	**18**	**14**	**20**	**12**	**18**	**22**	**11**	**27**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB87	**West**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**13**	**10**	**30**	**18**	**14**	**20**	**12**	**15**	**22**	**11**	**26**	**11**	**11**	**21**	**13**	**E1b1b V257 M81**
LB88	**West**	**13**	**24**	**13**	**10**	**14**	**15**	**11**	**14**	**11**	**30**	**18**	**14**	**20**	**12**	**19**	**20**	**11**	**23**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB89	**West**	**13**	**24**	**14**	**10**	**14**	**16**	**10**	**14**	**11**	**30**	**17**	**14**	**20**	**12**	**18**	**22**	**11**	**28**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB90	**West**	**13**	**24**	**13**	**10**	**14**	**16**	**10**	**13**	**11**	**29**	**17**	**14**	**20**	**12**	**19**	**22**	**11**	**27**	**11**	**11**	**22**	**12**	**E1b1b V257 M81**
LB91	**West**	**13**	**24**	**13**	**10**	**14**	**16**	**10**	**14**	**11**	**30**	**17**	**14**	**20**	**12**	**19**	**22**	**11**	**26**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB92	**West**	**13**	**24**	**12**	**10**	**14**	**16**	**10**	**14**	**11**	**30**	**17**	**14**	**20**	**12**	**19**	**22**	**10**	**26**	**11**	**11**	**21**	**13**	**E1b1b V257 M81**

LB93	**West**	**13**	**24**	**14**	**10**	**14**	**15**	**10**	**14**	**11**	**31**	**17**	**14**	**21**	**12**	**20**	**21**	**11**	**24**	**11**	**11**	**22**	**12**	**E1b1b V257 M81**
LB94	**Zou**	**15**	**21**	**15**	**11**	**15**	**17**	**15**	**13**	**15**	**29**	**15**	**14**	**20**	**11**	**17**	**18**	**12**	**22**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB95	**Zou**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**14**	**11**	**30**	**20**	**14**	**20**	**11**	**18**	**22**	**11**	**26**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB96	**Zou**	**13**	**24**	**14**	**10**	**14**	**15**	**10**	**14**	**11**	**31**	**17**	**14**	**21**	**11**	**18**	**21**	**11**	**25**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB97	**Zou**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**14**	**11**	**29**	**18**	**14**	**20**	**12**	**18**	**21**	**11**	**25**	**11**	**11**	**21**	**17**	**E1b1b V257 M81**
LB98	**Zou**	**12**	**25**	**13**	**10**	**13**	**15**	**10**	**14**	**11**	**31**	**15**	**14**	**20**	**12**	**20**	**23**	**11**	**27**	**11**	**11**	**21**	**12**	**E1b1b V257 M81**
LB99	**Zou**	**13**	**24**	**14**	**10**	**14**	**15**	**10**	**14**	**11**	**31**	**19**	**14**	**21**	**12**	**18**	**22**	**11**	**25**	**11**	**11**	**22**	**12**	**E1b1b V257 M81**
LB132	**Afr**	**14**	**21**	**15**	**11**	**15**	**17**	**12**	**12**	**11**	**29**	**16**	**14**	**22**	**11**	**16**	**18**	**12**	**25**	**11**	**11**	**21**	**12**	**E-M2 E1b1a V38 M4225**
LB133	**Afr**	**13**	**25**	**13**	**10**	**14**	**15**	**10**	**14**	**11**	**30**	**18**	**14**	**20**	**12**	**17**	**22**	**10**	**28**	**11**	**11**	**21**	**10.1**	**E-M2 E1b1a V38 M4225**
LB134	**Afr**	**13**	**24**	**13**	**10**	**14**	**14**	**10**	**14**	**11**	**31**	**18**	**14**	**20**	**11**	**19**	**22**	**11**	**27**	**11**	**11**	**21**	**12**	**E-M2 E1b1a V38 M4225**
LB135	**African**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**14**	**11**	**30**	**18**	**14**	**20**	**12**	**18**	**21**	**11**	**26**	**11**	**11**	**21**	**12**	**E-M2 E1b1a V38 M4225**
LB136	**Afr**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**14**	**11**	**30**	**18**	**14**	**20**	**12**	**18**	**23**	**11**	**26**	**11**	**11**	**21**	**12**	**E-M2 E1b1a V38 M4225**
LB137	**Afr**	**14**	**21**	**15**	**11**	**15**	**17**	**12**	**12**	**11**	**29**	**16**	**14**	**22**	**11**	**16**	**18**	**12**	**25**	**11**	**11**	**21**	**12**	**E-M2 E1b1a V38 M4225**
LB138	**Afr**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**14**	**11**	**30**	**18**	**14**	**20**	**12**	**18**	**23**	**11**	**26**	**11**	**11**	**21**	**12**	**E-M2 E1b1a V38 M4225**
LB139	**Afr**	**13**	**25**	**13**	**10**	**14**	**15**	**11**	**14**	**11**	**30**	**18**	**14**	**20**	**12**	**17**	**22**	**10**	**28**	**11**	**11**	**21**	**10.1**	**E-M2 E1b1a V38 M4225**
LB140	**Afr**	**13**	**24**	**13**	**10**	**14**	**14**	**10**	**14**	**11**	**31**	**18**	**14**	**20**	**11**	**19**	**22**	**11**	**27**	**11**	**11**	**21**	**12**	**E-M2 E1b1a V38 M4225**
LB14	**Afr**	**13**	**24**	**13**	**10**	**14**	**15**	**10**	**14**	**11**	**30**	**18**	**14**	**20**	**12**	**18**	**22**	**11**	**26**	**11**	**11**	**21**	**12**	**E-M2 E1b1a V38**

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LB142	Afr	13	24	13	10	14	15	10	14	11	30	18	14	20	12	18	23	11	26	11	11	21	12	E-M2 E1b1a V38 M4225
LB143	West	13	23	13	11	18	19	10	12	11	29	14	14	20	11	18	19	11	23	12	12	21	12	E-M78 E1b1b V65
LB144	West	13	22	13	11	18	19	11	12	11	29	14	14	20	11	18	20	11	23	12	12	21	12	E-M78 E1b1b V65
LB145	West	13	22	13	11	18	19	11	12	11	29	14	14	20	11	18	20	11	23	12	12	21	12	E-M78 E1b1b V65
LB146	Zou	12	23	10	11	14	14.3	11	13	11	30	19.2	14	20	11	16	17	11	26	13	11	21	9	J1a PF7257
LB147	East	13	23	14	11	14	19	13	13	11	29	18.2	14	19	11	16	18	10	24	12	11	20	8	J1a2a1a2 P58
LB148	Gha	12	23	14	11	14	19	11	14	11	30	17.2	14	20	11	17	17	11	25	12	11	21	9	J1a2a1a2 P58
LB149	Gha	12	22	14	12	14	20	11	13	11	30	18.2	14	20	11	18	18	11	26	14	11	21	9	J1a2a1a2 P58
LB150	Gha	12	22	14	12	14	20	11	13	11	30	18.2	14	20	10	17	19	11	26	13	11	21	9	J1a2a1a2 P58
LB151	Gha	12	23	14	12	14	20	11	12	11	29	19.2	14	19	11	19	13	11	26	13	11	21	9	J1a2a1a2 P58
LB152	West	13	21	15	11	15	18	12	13	11	31	18	14	21	11	13	18	12	27	11	11	24	14	J1a2a1a2 P58
LB153	West	12	22	14	12	14	21	11	13	11	30	18.2	14	20	11	17	18	11	26	13	11	21	9	J1a2a1a2 P58
LB154	West	12	22	14	12	14	21	11	13	11	30	18.2	14	20	11	17	18	11	26	13	11	21	9	J1a2a1a2 P58
LB15	West	13	23	14	11	14	19	13	13	11	29	18.2	14	19	11	16	18	11	24	12	11	20	9	J1a2a1a2 P58

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LB156	West	12	22	14	12	14	20	11	13	11	30	18.2	14	20	11	17	16	11	25	13	11	21	9	J1a2a1a2 P58
LB157	West	12	22	14	12	11	21	12	13	11	30	18.2	14	20	11	18	18	11	26	13	11	21	10	J1a2a1a2 P58
LB158	West	12	23	14	11	14	19	11	14	11	31	17.2	14	20	11	17	18	11	25	12	11	20	9	J1a2a1a2 P58
LB159	West	12	22	14	11	14	20	11	13	11	30	18.2	14	20	11	18	18	11	26	13	11	21	9	J1a2a1a2 P58
LB160	West	12	22	14	12	14	20	11	13	11	30	18.2	14	20	11	27	18	11	26	13	11	21	9	J1a2a1a2 P58
LB161	West	12	22	14	12	14	19	12	13	11	30	18.2	14	20	11	18	18	11	24	12	11	21	9	J1a2a1a2 P58
LB162	West	12	24	14	12	14	20	11	13	11	30	18.2	14	20	11	18	18	11	25	12	11	21	9	J1a2a1a2 P58
LB163	Zou	13	23	14	11	14	19	13	13	11	29	18.2	14	19	11	16	18	10	24	12	11	20	8	J1a2a1a2 P58
LB164	Zou	12	22	13	11	17	14	10	13	11	31	18.2	14	20	11	18	18	12	25	12	11	21	12	J1a2a1a2 P58
LB165	Zou	13	23	13	10	14	19	13	13	11	29	18.2	14	19	11	16	18	10	27	11	11	20	8	J1a2a1a2 P58
LB166	Zou	12	23	14	13	14	20	11	13	11	30	18.2	14	20	11	17	17	11	26	13	11	21	9	J1a2a1a2 P58
LB167	Zou	12	22	10	12	14	14	11	13	11.2	31	18.2	14	20	10	18	18	11	26	13	11	21	9	J1a2a1a2 P58
LB168	Zou	12	22	14	12	14	20	11	13	11	31	18.2	14	20	11	17	18	12	26	13	11	21	9	J1a2a1a2 P58
LB16	Zou	12	23	14	12	14	20	11	13	11	30	18.2	14	20	11	18	19	11	26	13	11	21	9	J1a2a1a2 P58

9																							
LB170	Zou	12	23	10	11	14.2	15.2	11	13	11	30	19	14	20	11	16	17	10	26	13	11	21	9	J1a2a1a2 P58
LB171	Zou	12	22	13.2	12	14.2	16.2	11	13	11.2	30	18.2	14	20	11	17	16	10	25	13	11	21	10	J1a2a1a2 P58
LB172	Gha	12	23	14	11	14	19	11	14	11	30	17.2	14	20	11	17	17	11	25	12	11	21	9	J1a2a1a2 P58
LB173	Gha	12	22	14	12	14.2	20	11	13	11	30	18.2	14	20	11	18	18	11	26	14	11	21	9	J1a2a1a2 P58
LB174	Gha	12	22	14	12	14	20	11	13	11	30	18.2	14	20	10	17	19	11	26	13	11	21	10	J1a2a1a2 P58
LB175	Gha	12	23	14	12	14	20	11	12	11	29	19.2	14	19	11	19	13	11	26	13	11	21	9	J1a2a1a2 P58
LB176	West	13	21	15	11	15	18	12	13	11	31	18	14	21	11	13	18	12	27	11	11	24	14	J1a2a1a2 P58
LB177	West	12	22	14	12	14	21	11	13	11	30	18.2	14	20	11	17	18	11	26	13	11	21	9	J1a2a1a2 P58
LB178	West	12	22	14	12	14	21	11	13	11	30	18.2	14	20	11	17	18	11	26	13	11	21	9	J1a2a1a2 P58
LB179	West	13	23	14	11	14	19	13	13	11	29	18.2	14	19	11	16	18	11	24	12	11	20	9	J1a2a1a2 P58
LB180	West	12	22	14	12	14	20	11	13	11	30	18.2	14	20	11	17	16	11	25	13	11	21	9	J1a2a1a2 P58
LB181	West	12	22	14	12	11	21	12	13	11	30	18.2	14	20	11	18	18	11	26	13	11	21	10	J1a2a1a2 P58
LB182	West	12	23	14	11	14	19	11	14	11	31	17.2	14	20	11	17	18	11	25	12	11	20	9	J1a2a1a2 P58
LB18	West	12	22	14	12	14	20	11	13	11	30	18.2	14	20	11	18	18	11	26	13	11	21	9	J1a2a1a2 P58

3																							
LB184	West	12	22	14	12	14	20	11	13	11	30	18.2	14	20	11	18	18	11	26	13	11	21	9	J1a2a1a2 P58
LB185	West	12	22	14	12	14	19	11	13	11	30	18.2	14	20	11	18	18	11	25	12	11	20	9	J1a2a1a2 P58
LB186	West	12	24	14	12	14	20	11	13	11	30	18.2	14	20	11	18	18	11	25	12	11	21	9	J1a2a1a2 P58
LB187	Zou	13	23	14	11	14	19	13	13	11	29	18.2	14	19	11	16	18	10	24	12	11	20	8	J1a2a1a2 P58
LB188	Zou	12	22	13	11	17	14	10	13	11	31	18.2	14	20	11	18	18	12	25	12	11	21	12	J1a2a1a2 P58
LB189	Zou	13	23	13	10	14	19	13	13	11	29	18.2	14	19	11	16	18	10	27	11	11	20	8	J1a2a1a2 P58
LB190	Zou	12	23	14	13	14	20	11	13	11	30	18.2	14	20	11	17	17	11	26	13	11	21	9	J1a2a1a2 P58
LB191	Zou	12	22	10	12	14	14	11	13	11.2	31	18.2	14	20	10	18	18	11	26	13	11	21	9	J1a2a1a2 P58
LB192	Zou	12	22	14	12	14	20	13	13	11	31	18.2	14	20	11	17	18	12	26	13	11	21	8	J1a2a1a2 P58
LB193	Zou	12	23	14	12	14	20	11	13	11	30	18.2	14	20	11	18	19	11	26	13	11	21	9	J1a2a1a2 P58
LB194	Zou	12	23	13.2	11	14.2	17.2	11	14	11.2	31	17.2	14	20	11	17	18	10	25	12	11	20	8	J1a2a1a2 P58
LB195	Zou	12	22	13.2	12	14.2	15.2	11	14	11.2	30	18.2	14	20	11	17	16	10	25	13	11	21	8	J1a2a1a2 P58
LB196	Zou	13	22	13.2	12	14.2	15.2	11	13	11.2	30	18.2	14	20	10	17	19	10	26	13	11	21	10	J1a2a1a2 P58
LB19	Zou	12	23	13.2	12	14.2	20.2	11	13	11.2	30	18.2	14	20	11	17	19	10	26	12	11	21	9	J1a2a1a2 P58

7																							
LB198	Zou	12	23	13.2	13	14.2	20.2	11	13	11.2	30	18.2	14	20	11	17	17	10	27	13	11	21	9	J1a2a1a2 P58
LB199	Zou	12	23	10	11	14.2	15.2	11	13	11	30	19	14	20	11	16	17	10	26	13	11	21	9	J1a2a1a2 P58
LB200	Zou	12	22	13.2	12	14.2	16.2	11	13	11.2	30	18.2	14	20	11	17	16	10	25	13	11	21	9	J1a2a1a2 P58
LB201	Zou	12	23	13.2	11	14.2	18.2	11	13	11.2	29	13	15	19	11	17	15	9	23	12	11	23	10	J2a1 M319
LB202	Zou	12	24	13.2	11	12.2	20.2	11	13	11	31	16	15	21	11	16	17	9	22	12	11	22	10	J2a1 M319
LB203	Zou	12	23	13.2	11	14.2	18.2	11	13	11.2	29	13	15	19	11	17	15	9	23	12	11	23	10	J2a1 M319
LB204	Zou	12	24	13.2	11	12.2	20.2	11	13	11	31	16	15	21	11	16	17	9	22	12	11	22	10	J2a1 M319
LB205	Zou	12	24	13.2	11	12.2	20.2	11	13	11	31	16	15	21	11	16	17	9	22	12	11	22	10	J2a1 M319
LB206	Zou	12	23	13.2	11	14.2	18.2	11	13	11.2	29	13	15	19	11	17	15	9	23	12	11	23	10	J2a1 M319
LB207	Zou	12	23	14	11	14	18	13	13	11	29	13	15	19	11	17	15	9.2	23	12	11	23	10	J2a1 PF5191
LB208	Zou	12	23	14	11	14	18	13	13	11	29	13	15	19	11	17	15	9.2	23	12	11	23	10	J2a1 PF5191
LB209	West	12	24	14	11	14	20	11	13	11	31	16	15	21	11	16	17	9.2	22	12	11	22	10	J2a1 M67 S25258
LB210	West	12	23	14	11	14	18	13	13	10	29	13	15	19	12	17	15	10	23	12	11	23	10	J2a1 PF51
LB21	West	12	23	14	11	14	18	13	13	11	29	13	15	19	11	17	15	10	23	12	11	23	10	J2a1 PF51

1																							
LB212	Naf	11	23	15	11	13	19	12	14	14	30	17	16	19	11	18	14	11	25	13	12	21	11	L1b M317
LB213	Naf	11	23	15	11	13	19	12	15	14	30	18	16	18	11	18	14	11	25	13	11	22	11	L1b M317
LB214	West	11	23	15	11	13	19	12	14	14	30	18	16	19	11	18	14	11	26	13	12	21	11	L1b M317
LB215	Gha	13	23	14	13	14	15	13	14	13	30	16	14	19	12	20	21	13	21	12	12	23	12	R1b V88
LB216	Gha	13	23	14	13	14	16	10	14	11	30	16	14	19	12	20	21	13	22	12	11	23	12	R1b V88
LB217	Gha	14	25	15	12	14	16	12	14	13	30	18	14	19	13	17	16	11	22	12	12	23	11	R1b V88
LB218	Zou	13	24	14	10	14	16	10	14	11	31	18	14	20	11	17	21	11	25	11	11	21	12	R1b V88
LB219	Gha	13	24	14	11	14	17	12	14	13	31	17	14	19	11	18	17	9	22	13	12	21	12	T PF5633 CTS11451

Table 20. Allele frequency and diversity parameter for Y- STR data

	DYS393	DYS19	DYS391	DYS385a	DYS385b	DYS439	DYS389I	DYS392	DYS389II	DYS448	YGATAH4	DYS438	DYS635	DYS437	DYS458	DYS390	
4																	
5																	
6																	
7																	
8								0.00134			0.00402	0.00134					
9			0.2784			0.00402					0.00535	0.19411					
10	0.00134	0.0054	0.3133	0.00134		0.3668	0.00134	0.01874			0.02677	0.60643					
11	0.01606	0.0054	0.3708	0.0241	0.00268	0.3855	0.01071	0.8715			0.5274	0.14726					
12	0.391	0.0094	0.0335	0.0442	0.00535	0.16198	0.09638	0.03345			0.3963	0.04685			0.00134		
13	0.5114	0.4029	0.00401	0.5636	0.03079	0.07898	0.5863	0.04149			0.03882	0.002677		0.00535	0.00669		
14	0.0683	0.4538		0.1834	0.10442	0.00134	0.2972	0.28112			0.00134	0.00134		0.8862	0.0589		
15	0.00803	0.0884		0.0522	0.2557	0.00134	0.00803	0.00535						0.06961	0.06292		
16	0.00134	0.0348		0.0522	0.08567									0.03615	0.08434		
17	0.00268			0.0589	0.06024					0.00401				0.002677	0.25569		
18				0.0174	0.11512					0.00937					0.41901		
19				0.00937	0.25033					0.12048			0.002677		0.01017		
20				0.00402	0.06291					0.7456			0.08701		0.017403		
21					0.01874					0.10174			0.71352		0.00402	0.0415	
22					0.00535					0.01339			0.100401			0.1234	
23					0.00535					0.00268			0.06291			0.3855	
24										0.00268			0.03213				
25																	
26																0.0054	
27									0.008032								

28									0.06961								
29									0.38955								
30									0.41231								
31									0.10843								
32									0.01071								
	0.5564	0.646263	0.767891	0.634194	0.934621	0.759819	0.484024	-1.00024	0.718705	0.01532	0.515195	0.494216	0.175091	-0.86987	0.833795	0.746591	PD
	0.28943	0.330887	0.400708	0.34585	0.610807	0.399104	0.268925	0.024511	0.372733	0.157595	0.27263	0.280133	0.193686	0.041961	0.475156	0.392185	PE
	0.496479	0.54893	0.621472	0.607046	0.811659	0.625633	0.489542	0.032782	0.598956	0.390623	0.473178	0.523491	0.444839	0.198729	0.713706	0.621719	PIC
	0.580591	0.622565	0.685706	0.636858	0.831478	0.684352	0.558454	0.158237	0.661469	0.418917	0.562525	0.570673	0.468239	0.208461	0.744043	0.678465	H

PD, power of discrimination; PE, probability of exclusion; PIC, polymorphism information content

Table 21. Worldwide populations used in the comparative analysis

Population	N	Reference			
Libyan Tuareg	129	Ottoni et al., 2009	Eastern Arab	43	This study
Takrouna	33	S. Frigi, et al. 2017	Jerbian Berber	30	Loueslati et al., 2006
Turkey	290	McEvoy et al. 2004	Kurdistan	53	McEvoy et al. 2004
Kuwait	94	(Theyab et al., 2012)	African Libyan	32	This study
Jerbian Arab	29	Cherni et al., 2005	Sejnane	47	Plaza et al., 2003
Siwa Berber	78	Coudray et al., 2009	Mauritanians	64	Plaza et al., 2003
Kesra Berber	90	Cherni et al., 2005	Western Arab	125	This study
Skhira Berber	20	Cherni et al., 2009	Sejnane	47	Plaza et al., 2003
Figuig Berber	94	Coudray et al., 2009	Saharaui	56	Plaza et al., 2003
Chnenni- Berber	53	Fadahlaoui-Zid et al., 2004	Jeriba	85	Cherni et al., 2009
Mozabites Berber	85	Macaulay et al., 1999			
Kalaat El Andalous	29	Cherni et al., 2009			
Matmata Berber	49	Fadahlaoui-Zid et al., 2004			
Testour	50	Cherni et al., 2009			
Kalat Al alia	48	Plaza et al., 2003			
Souss Berber	50	Brakez et al., 2001			
Asni Berber	53	Coudray et al., 2009			
Bouhria Berber	70	Coudray et al., 2009			
Portuguese	1591	Barral-Arca et al. 2017			
Algerians	47	Plaza et al., 2003			
Moroccan Berber	64	Plaza et al., 2003			
Slouguia	28	Cherni et al., 2009			

Andalusia	1253	Barral-Arca al., 2016			
Sened Berber	53	Fadahlaoui-Zid et al., 2004			