1.

For tree reconstruction I used MAFFT and IQtree (I also added Neanderthal for rooted tree):

```
cat * > people_mt.fasta
awk '/^>/ {for(i=1; i<=NF; i++) if($i=="haplogroup") print $1 "_" $(i+1)} !/^>/ {print}'
people_mt.fasta > people_mt2.fasta
cat people_mt2.fasta 'GoyetQ57-2 Neanderthal.fasta' > rooted_mt.fasta
mafft --auto rooted_mt.fasta > rooted_mt_aln.fasta
trimal -in rooted_mt_aln.fasta -out rooted_mt_aln_trimmedML.fasta -keepheader
-automated1
./iqtree-1.6.12-Linux/bin/iqtree -s rooted_mt_aln_trimmedML.fasta -bb 1000 -alrt 1000 -pre
rooted_mt
```
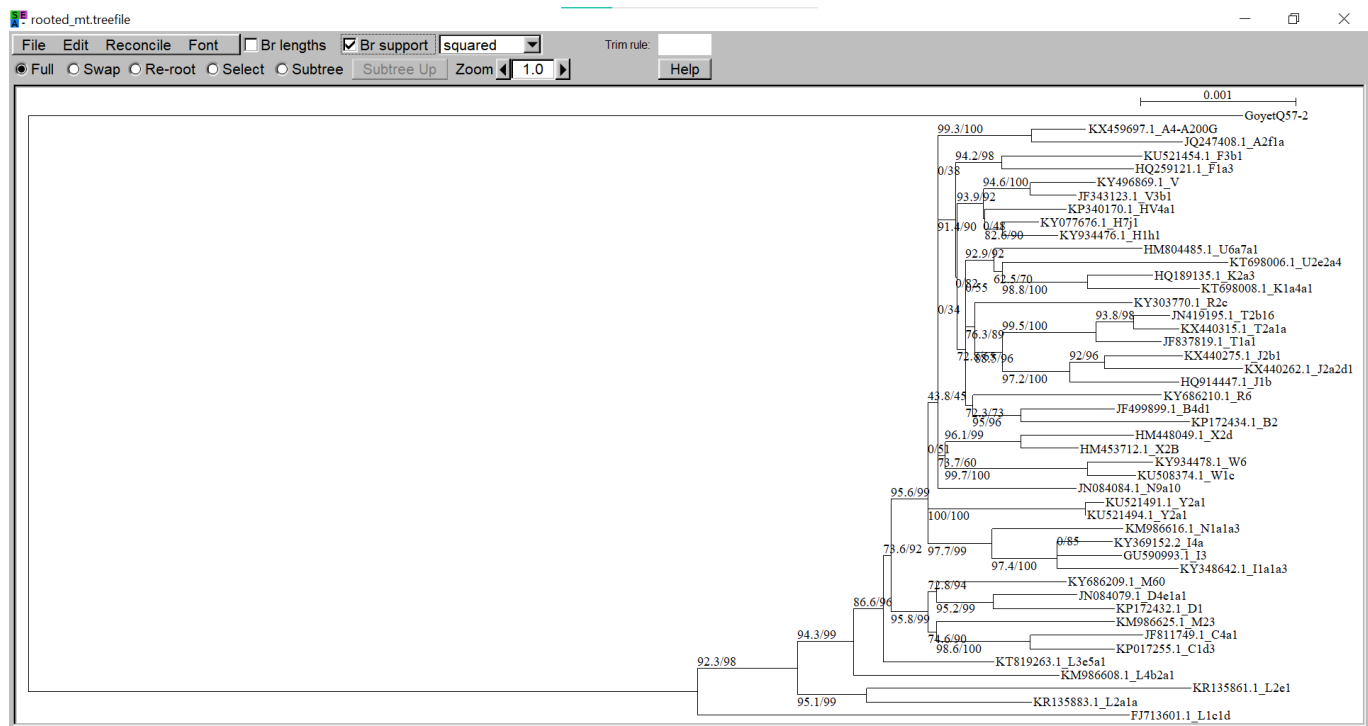

2.

I took mtDNA mutation rate (average 3.12 × 10–8) from that article: [Human molecular evolutionary rate, time dependency and transient polymorphism effects viewed through ancient and modern mitochondrial DNA genomes | Scientific Reports](#)

"The observed evolutionary rates range from **4.33 × 10–8** (95% CI 3.90–4.82 × 10–8) mutations per site per year (msy) for the most recent period to **1.91 × 10–8** (95% CI 1.72–2.10 × 10–8) msy for the oldest period (40,160 ± 4658 ya). "

```
cat 'L1c1d Central African.fasta' 'J2a2d1 Tunisian.fasta' > formega.fasta
mafft --auto formega.fasta > formega_aln.fasta
```

I used 'L1c1d Central African.fasta' and 'J2a2d1 Tunisian.fasta' that have the max distance on the tree between each other. MEGA software was used to calculate pairwise distances.

M11: Pairwise Distances (formega_aln.fasta)

	1	2
1. FJ713601.1 Homo sapiens haplogroup L1c1d mitochondrion complete genome		
2. KX440262.1 Homo sapiens isolate JT76 haplogroup J2a2d1 mitochondrion complete genome	0.00573	

[1,1] (FJ713601.1 Homo sapiens haplogroup L1c1d mitochondrion complete genome-FJ713601.1 Homo sapi

0.00573 / 3.12 × 10–8 = 183,654 years - age of the mitochondrial Eve

To estimate the age of the most recent ancestor of all non-Africans I used 'U2e2a4 Serbian.fasta' and 'L4b2a1 Yemeni.fasta'.

cat 'U2e2a4 Serbian.fasta' 'L4b2a1 Yemeni.fasta' > formeganon.fasta
mafft --auto formeganon.fasta > formeganon_aln.fasta

M11: Pairwise Distances (formeganon_aln.fasta)

	1	2
1. KT698006.1 Homo sapiens isolate 52 Sb haplogroup U2e2a4 mitochondrion complete genome		
2. KM986608.1 Homo sapiens isolate Y453 haplogroup L4b2a1 mitochondrion complete genome	0.00326	

[1,1] (KT698006.1 Homo sapiens isolate 52 Sb haplogroup U2e2a4 mitochondrion complete genome-KT698006.1 Homo sapiens isolate 52 Sb haplogroup U2e2a4 mitochondrion complete genome) / Nucleotide:

0.00326 / 4.33 × 10–8 = 75,288 years
or if take lower mutation rate 0.00326 / 3.12 × 10–8 = 104,487 years
- age of the most recent ancestor of all non-Africans

3.

cat * > denisova_mt.fasta
cat * > neanderthal_mt.fasta
cat people_mt.fasta denisova_mt.fasta neanderthal_mt.fasta > mixed_mt.fasta

mafft --auto mixed_mt.fasta> mixed_mt_aln.fasta
trimal -in mixed_mt_aln.fasta -out mixed_mt_aln_trimmedML.fasta -keepheader -automated1
./iqtree-1.6.12-Linux/bin/iqtree -s mixed_mt_aln_trimmedML.fasta -bb 1000 -alrt 1000 -pre mixed_mt
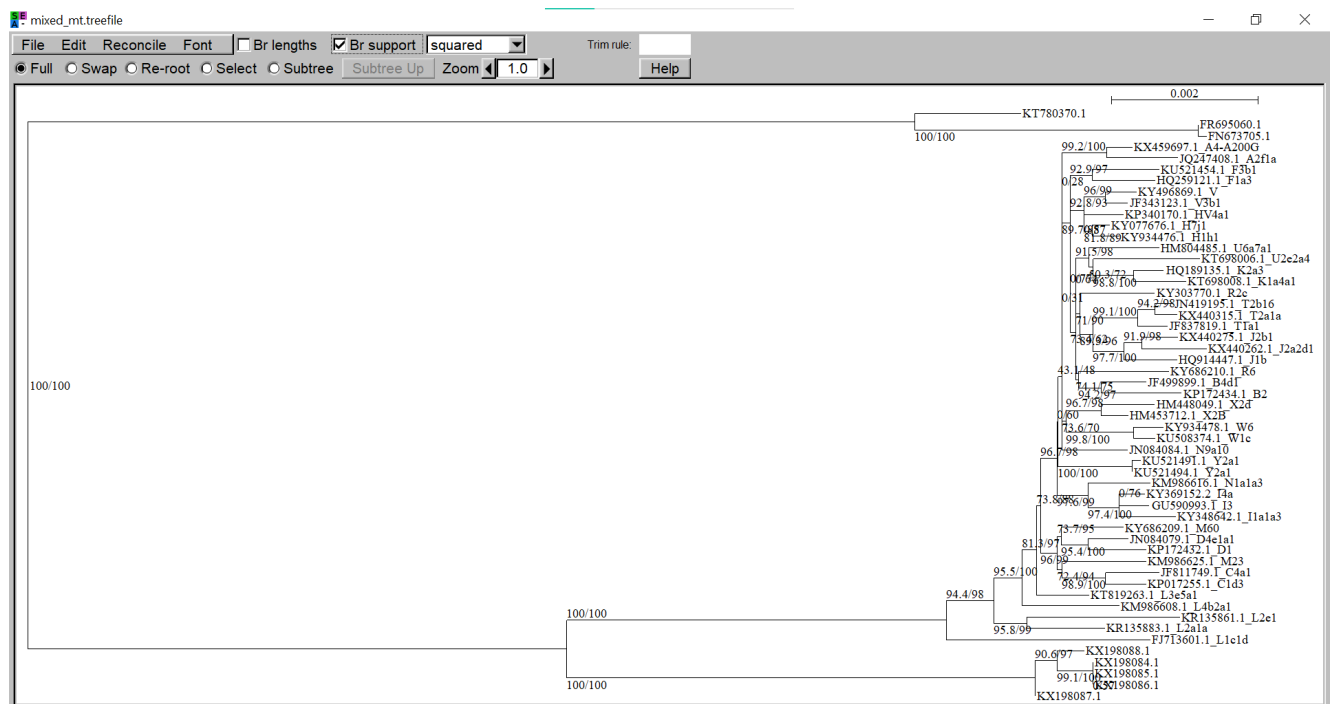
As we can see 5 Neanderthals and 3 Denisovans are separated from the modern humans. Moreover, according to the tree Neanderthals are closer to modern humans.

Now it's time to estimate the age of the most recent Neanderthal-modern human ancestor:

```
cat 'J2a2d1 Tunisian.fasta' 'GoyetQ374a-1 Neanderthal.fasta' > formega.fasta
mafft --auto formega.fasta > formega_aln.fasta
```


0.0133 / 3.12 × 10–8 = 426,282 years
or if take lower mutation rate 0.0133 / 2.5 × 10–8 = 532,000 years
- age of the most recent Neanderthal-modern human ancestor

0.022 / 3.12 × 10–8 = 705,128 years
or 0.022 / 2.5 × 10–8 = 880,000 years
- age of the most recent Neanderthal-Denisovans ancestor

To sum up, according to my results, the most recent ancestor of all non-Africans

lived about 100,000 to 70,000 years ago. Mitochondrial Eve is estimated to have lived around 180,000 years ago, and the most recent common ancestor of Neanderthals and modern humans existed about 400,000 to 500,000 years ago. Neanderthals and Denisovans separated approximately between 500,000 and 880,000 years ago.