

# mtDNA Diversity of Some Turkish Native Goat Breeds



Bengi ÇINAR KUL, Okan ERTUĞRUL  
Ankara University,  
Department of Genetics,  
Faculty of Veterinary Medicine, Turkey



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- Domestication of the animals, considered as one of the most important milestones of modern human life, began about 11,000 years ago in the Neolithic age on the lands known as "Fertile Crescent" (Zeder, 2008).
- The four livestock species:





In comprehensive studies focusing on the goat domestication, Cytochrome *b* and D-loop regions located on mtDNA have been frequently used (Luikart et al., 2001; Naderi et al., 2007).

In these studies, more sample analyses from domestication centers, such as Turkey... these species have distributed to European zones across Anatolia... (MacHugh and Bradley, 2001; Naderi et al., 2008; Zeder, 2008).





- Turkish native goat breeds, have an important position in domestication history, besides they are valuable genetic resources for the ecosystem and economy. In this study, it is aimed to unveil the domestication history of the goat and to explain the mtDNA diversity of these breeds.
- For this purpose;  
D-loop and Cytochrome *b* regions were sequenced and analyzed from the Angora, Honamli, Kilis, Hair and Norduz goat breeds.



Louvre Museum. R.M.N./P.  
Bernard ve Oi-Cheong Lee





# Materials and Methods

## Sampling and DNA isolation procedures

252 blood samples from 5 native breeds of goats.

Norduz



n≈50 each breed

Angora



Kilis



5

Honamlı



Hair







**In order to represent a reliable level of genetic diversity in the breeds that;**

- regions, families, genders, ages, health conditions.
- geographic coordinates were recorded using GPS.



## DNA Isolation

- ✓ Phenol/chloroform method was used.

## DNA checking

- ✓ Concentration, purity and degradation of DNAs were checked by Nanodrop and agarose gel electrophoresis.







## *Amplification and sequencing*

The complete **D-loop** was amplified by using primers suggested by Sultana et al. (2003) and **Hypervariable region-1 (HV-1)** was sequenced by using two internal primers described by Naderi et al (2007).

**Cytochrome b** regions were amplified and sequenced (Approx. 1140 bp) by using primers designed in the present study, in order to further investigate the introgression time of haplogroups.

Sequenced on **both strands** by using an ABI 3130 autoanalyzer.





## Data and phylogenetic analyses



- Sequences were **aligned and edited** (BioEdit V7.0.9.1)



- **Polymorphisms** were analyzed (DnaSP V5)



- **Phylogenetic trees** (ML & NJ) were constructed (PhyML V3.0 and MEGA 4.0)



- **F statistics** were assessed (MEGA 4.0 )



- **MJ network** was generated (Network V.4.516)

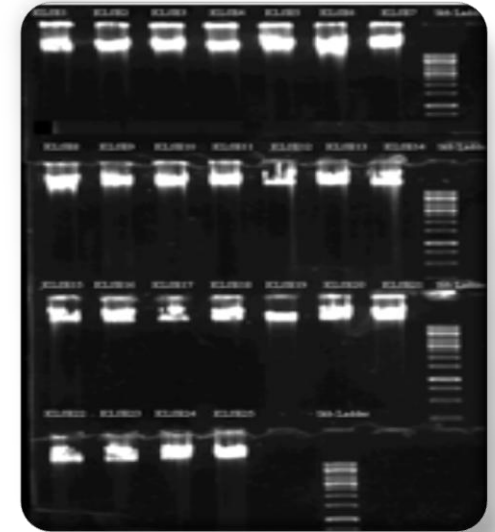
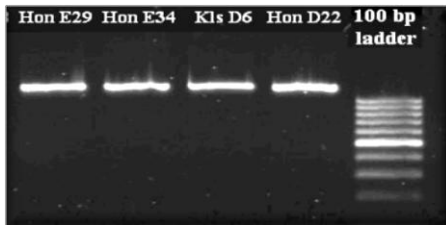
- **Neutral theory** was tested (DnaSP V5)



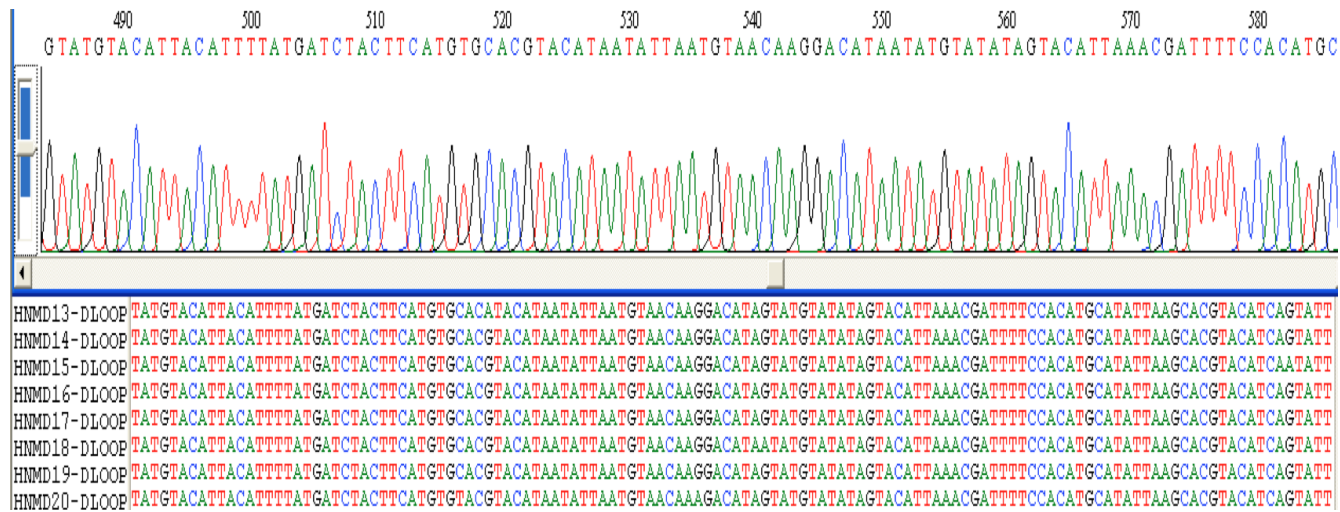
# Results and Discussion

## *DNA isolation and sequencing*

DNA isolation, quantification and PCR reactions were successfully performed for all samples.







According to results of D-loop sequences, duplication insertions (77bp) observed on two individuals from different breeds were quite remarkable.



**Similar mutations have been reported by several researchers** (Sultana et al., 2004; Pereira et al., 2005; Naderi et al., 2007)

It is interesting that...

Same mutation;

Different haplogroups

Different breeds



# *Structure of the populations*

Domestication region  genetic diversity 

(without selection and isolation)




Haplotype diversity  
Nucleotide diversity



## Statistical data based on the mtDNA diversity

Breed	N	Nh	$h \pm sd$	$\pi \pm sd$
Angora	50	42	0.993; $\pm 0,005$	0,0202; $\pm 0.00162$
Honamli	49	42	0.993; $\pm 0.006$	0.0233; $\pm 0.00187$
Kilis	51	48	0.998; $\pm 0.004$	0.0205; $\pm 0.00188$
Hair	53	48	0.996; $\pm 0.005$	0.0203; $\pm 0.00108$
Norduz	49	36	0.983; $\pm 0.008$	0.0192; $\pm 0.00101$
Total	252	208	0.9982; $\pm 0.0006$	0.0210; $\pm 0.00073$





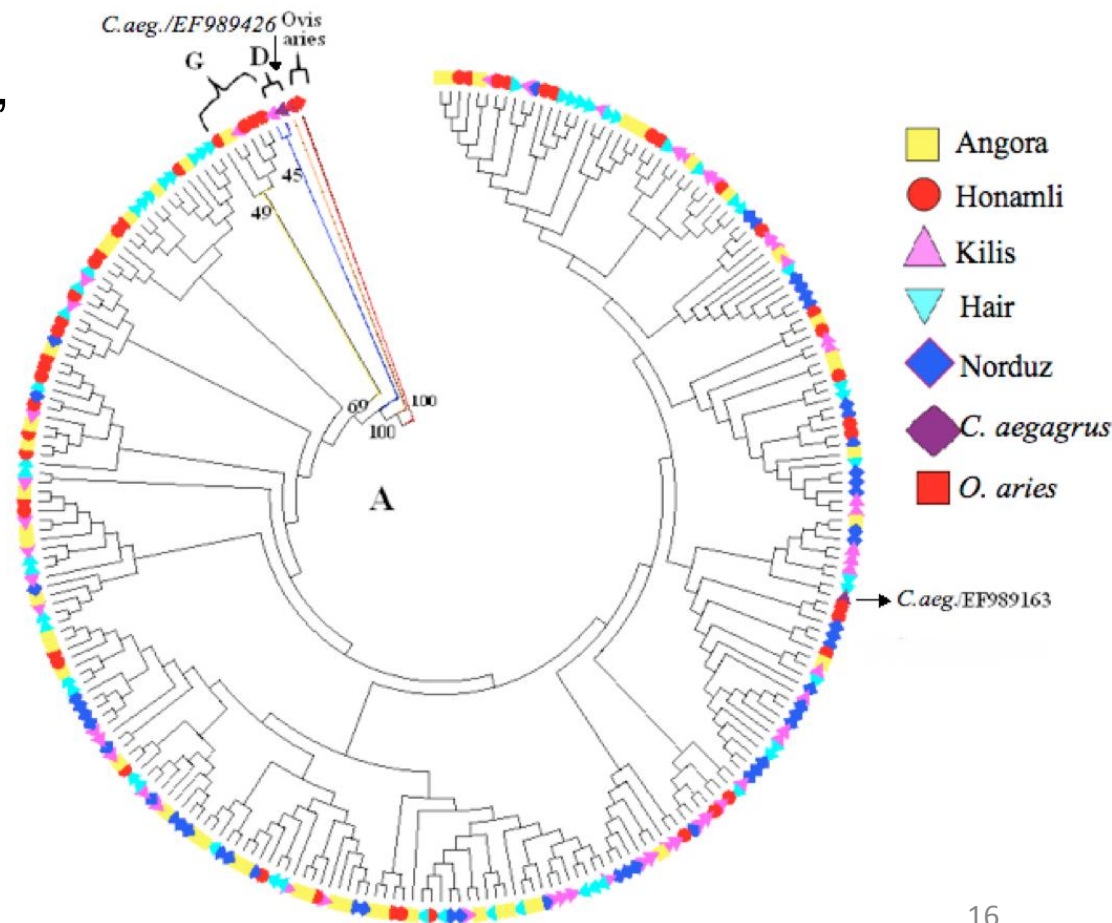
✓ **Turkey is one of the central localization for goat domestication...**

In terms of nucleotide and haplotype diversity, all groups **showed high values** as compared with mainly world goat breeds (Luikart et al., 2001; Joshi et al., 2004; Pereira et al., 2005; Naderi et al., 2007).

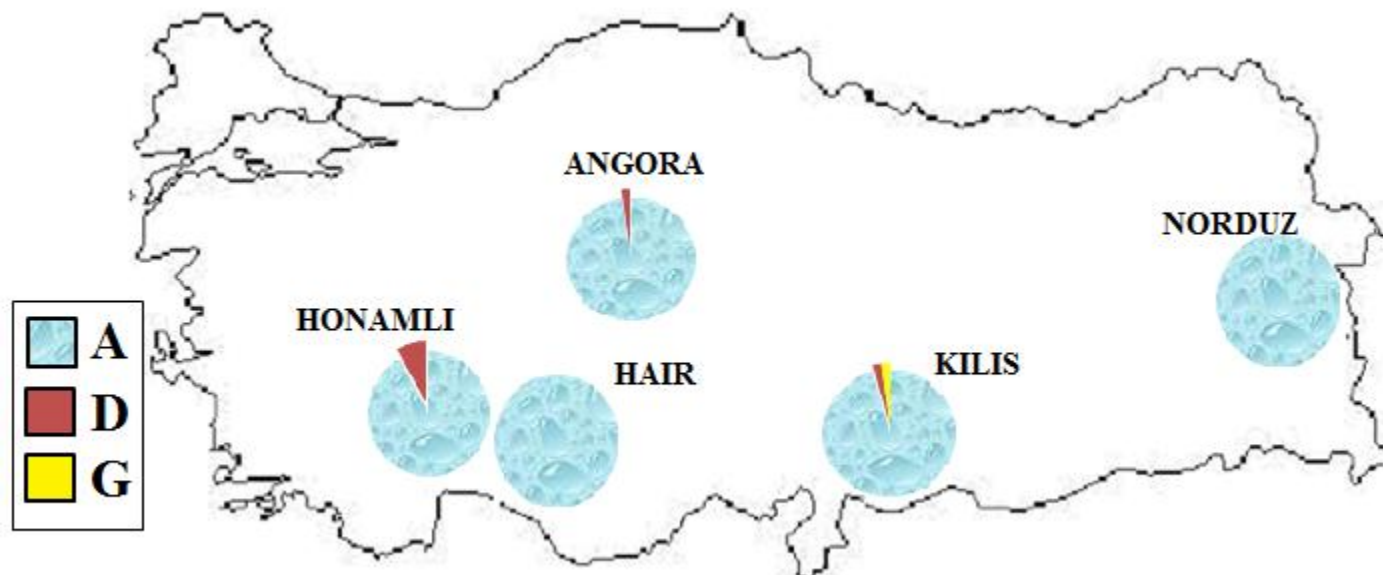
These results suggest that Turkey is **a central localization** for goat domestication in accordance with Naderi et al. (2008).

## ✓ Three distinct haplogroups...

Based on the phylogenetic trees, 245 of the animals were classified in **Haplogroup A**, which is thought to be the oldest haplogroup. Six goats were classified in **Haplogroup G** and one goat is classified in **Haplogroup D**.



Haplogroups	Angora	Kilis	Honamlı	Hair	Norduz
Haplogroup A	%98	%96	%92	%100	%100
Haplogroup D	-	%2	-	-	-
Haplogroup G	%2	%2	%8	-	-



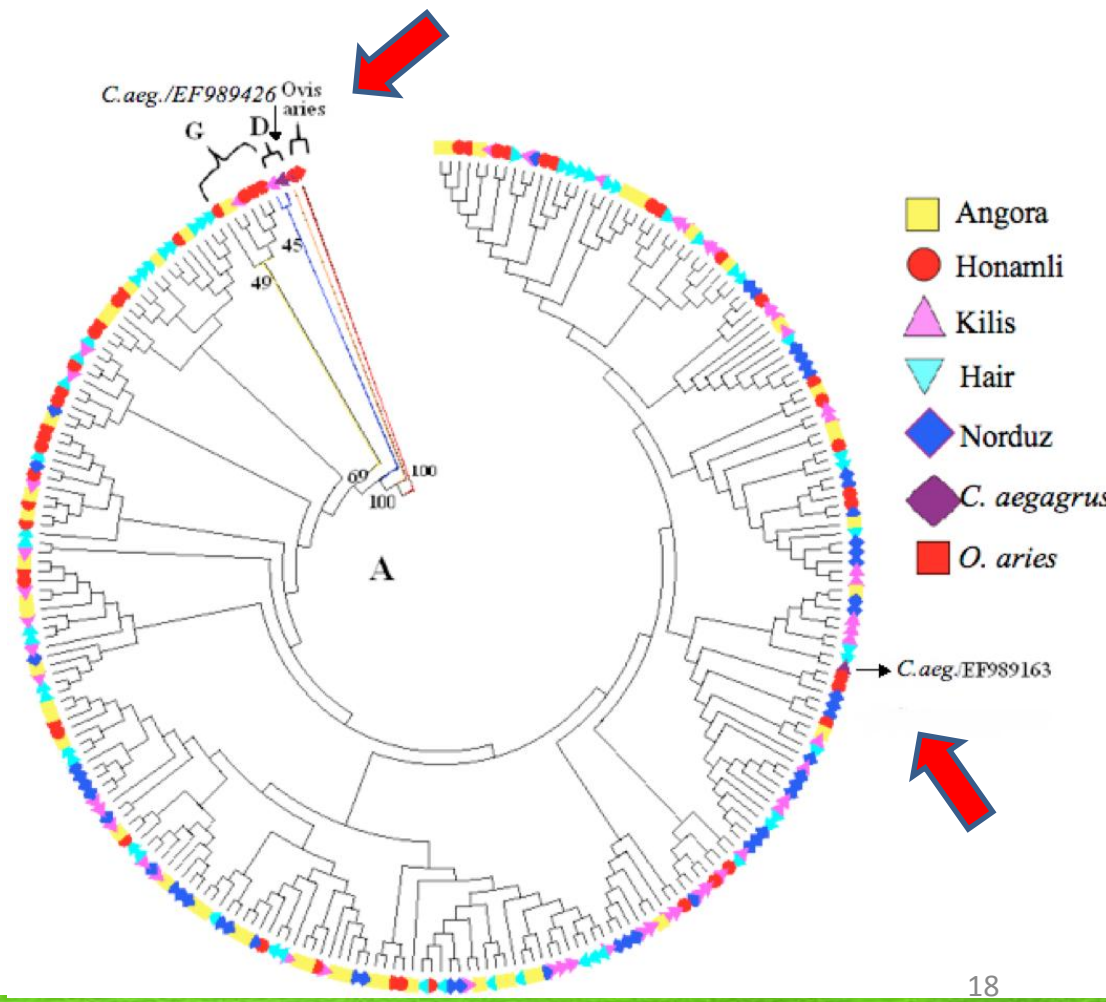
The haplogroups distribution of studied breeds.



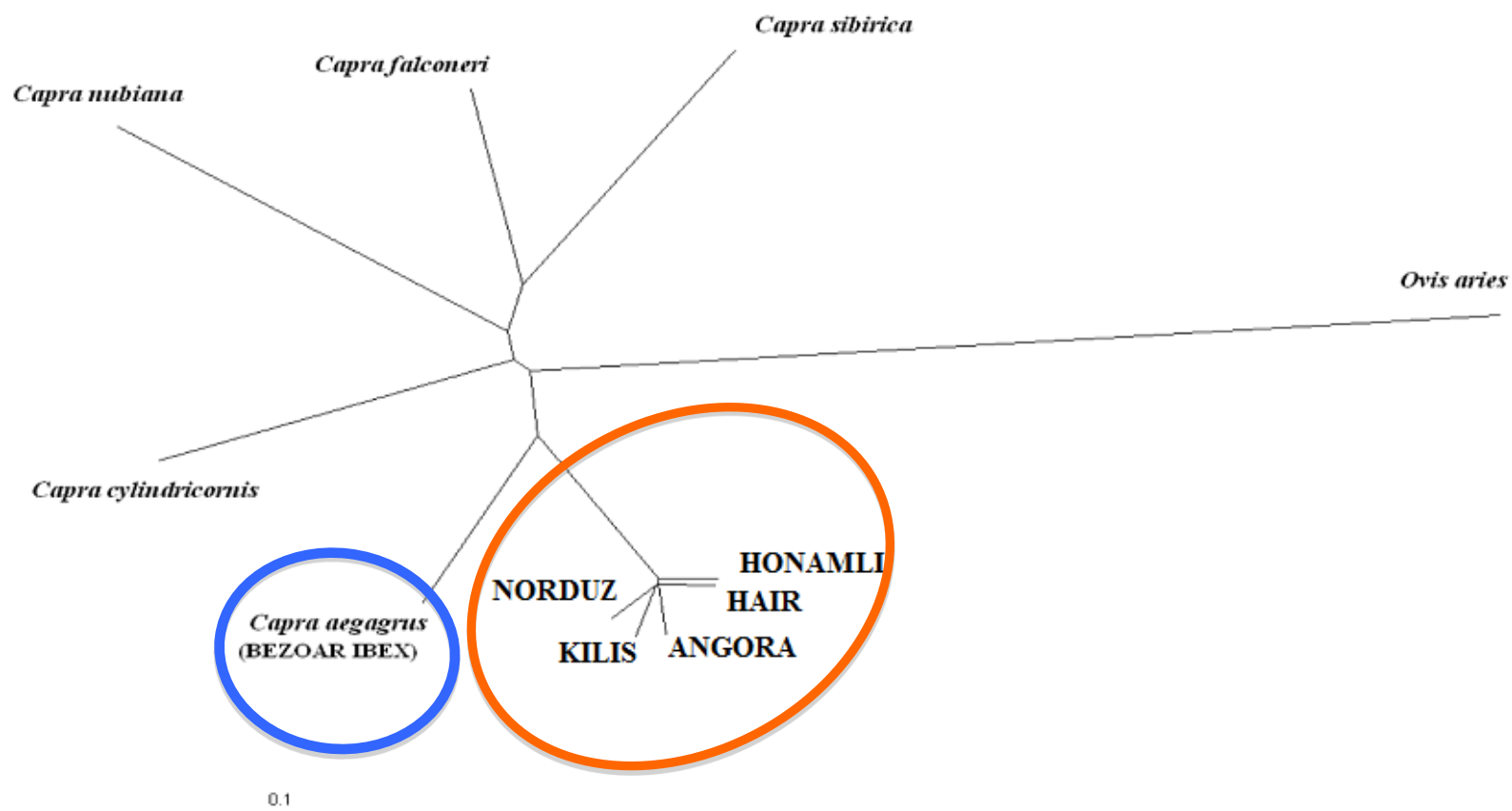
# History of the populations

✓ Closeness with wild goats...

Some of the goats were identified on the **same branch** with two wild goats (*C. aegagrus*, EF989426 & EF989163).

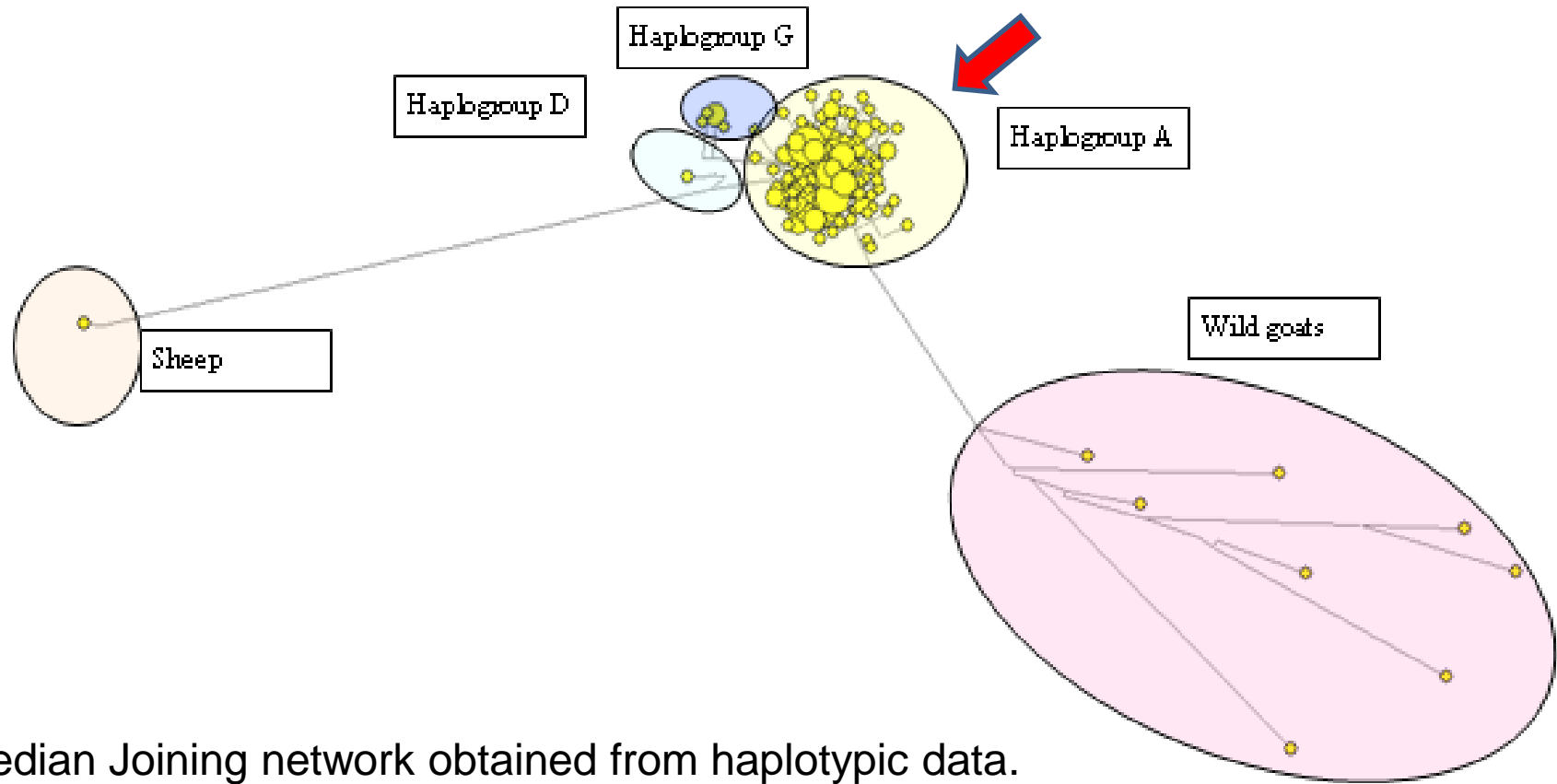


- According to pairwise  $F_{ST}$  distances, all breeds have been involved on the same branch with *Capra aegagrus*.





✓ Haplogroup A has a star-like pattern...



Median Joining network obtained from haplotypic data.

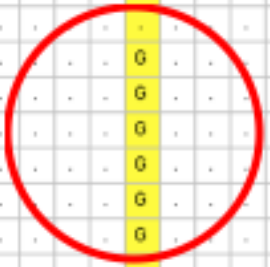




# ✓ Unique mutation for G haplogroup...

	A	A	C	A	T	G	C	A	T	A	T	C	C	G	C	C	A	T	T	A	G	A	T	C	A	C	G	A	G	C	T	T	A	T	T	G
✓ 18. HNMD14DLOOP (HONAM)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.
✓ 19. HNMD15DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.
✓ 20. HNMD16DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
✓ 21. HNMD17DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
✓ 22. HNMD18DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
✓ 23. HNMD19DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
✓ 24. HNMD20DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	T	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
✓ 25. HNMD21DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
✓ 26. HNMD23DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.
✓ 27. HNMD24DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.
✓ 28. HNMD25DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.
✓ 29. HNME26 DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.
✓ 30. HNME27 DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.
✓ 31. HNME30 DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.
✓ 32. HNME31 DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.
✓ 33. HNME32 DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.
✓ 34. HNME33 DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.
✓ 35. ANKD14DLOOP (ANKARA)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	G	.	C
✓ 36. KLSDB D LOOP (KILIS)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	G	.	C
✓ 37. HNMD22DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	G	.	C
✓ 38. HNME28 DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	G	.	C
✓ 39. HNME29 DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	G	.	C
✓ 40. HNME34 DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	G	.	C
✓ 41. HNME35 DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	G	.	C
✓ 42. HNME36 DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	G	.	C
✓ 43. HNME37 DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	G	.	C
✓ 44. HNME38 DLOOP (HONAM)	.	.	.	.	.	.	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	G	.	C

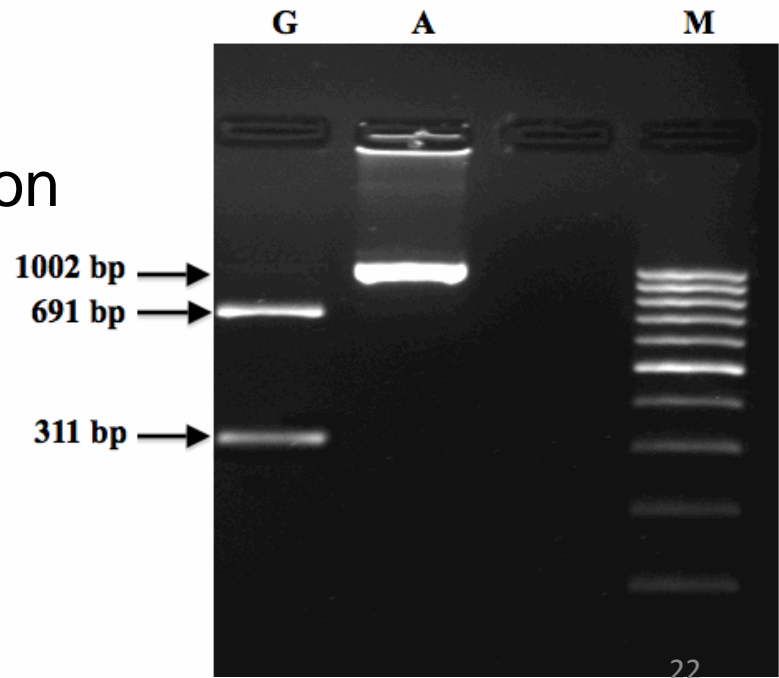
**A16071G**





# ✓ A novel method for determination of G haplogroup

- For the determination of this haplogroup (G), a novel method was introduced.
- In this wise, two segments were harvested via an enzymatic digestion process of D-loop region.



## ✓ Population expanded has been exposed...

- Fu's Fs and Tajima's D values are provided on Table.

Breed	N	Nh	$h \pm sd$	$\pi \pm sd$	Fu's Fs	Tajima's D
Angora	50	42	0.993; $\pm 0,005$	0,0202; $\pm 0.00162$	-32.968	-1.57723*
Honamli	49	42	0.993; $\pm 0.006$	0.0233; $\pm 0.00187$	-29.266	-1.25471
Kilis	51	48	0.998; $\pm 0.004$	0.0205; $\pm 0.00188$	-47.991*	-1.85794**
Hair	53	48	0.996; $\pm 0.005$	0.0203; $\pm 0.00108$	-44.975	-1.46930
Norduz	49	36	0.983; $\pm 0.008$	0.0192; $\pm 0.00101$	-20.170	-1.30735
Total	252	208	0.9982; $\pm 0.0006$	0.0210; $\pm 0.00073$		

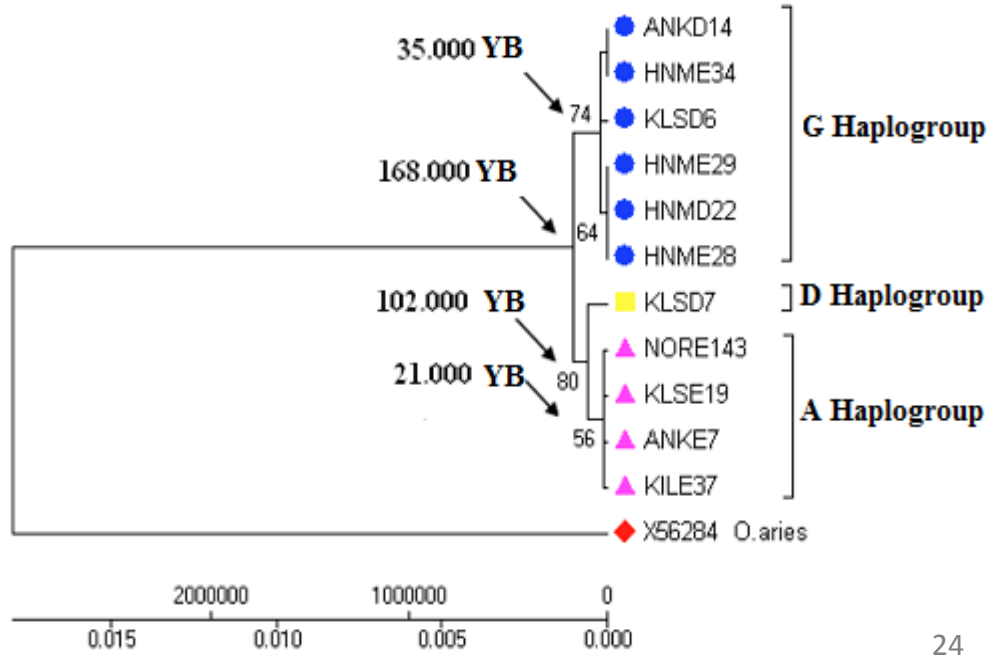
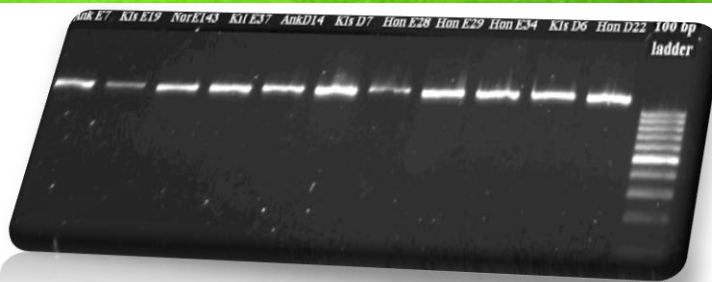
\*, P < 0.10; \*\*, P < 0.05





# ✓ TMRCA was established...

The TMRCA based on cytochrome *b* distances for Haplogroups were calculated to be 168,000 years before and G was found to be the oldest haplogroup among all.



TMRCA; Nei's method ( $D=2urt$ )

# Conclusion

Phylogenetic studies display domestication regions of species and intercontinental migration routes.

As an example...



PROCEEDINGS  
— OF —  
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SOCIETY

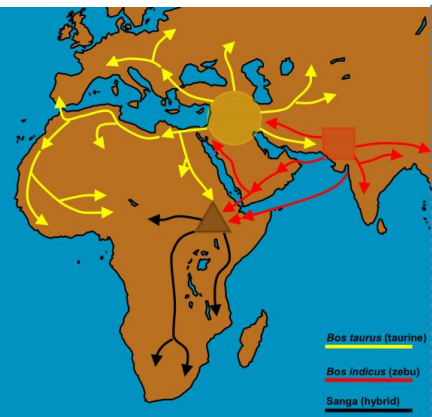
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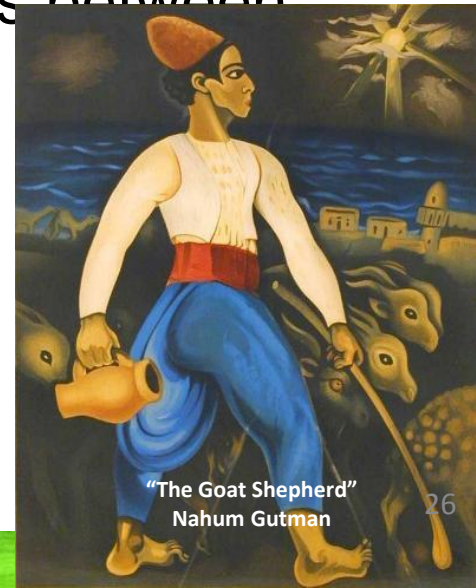
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## The mystery of Etruscan origins: novel clues from *Bos taurus* mitochondrial DNA

Marco Pellecchia<sup>1</sup>, Riccardo Negrini<sup>1</sup>, Licia Colli<sup>1</sup>, Massimiliano Patrini<sup>1</sup>,  
Elisabetta Milanese<sup>1,†</sup>, Alessandro Achilli<sup>2</sup>, Giorgio Bertorelle<sup>3</sup>,  
Luigi L. Cavalli-Sforza<sup>4</sup>, Alberto Piazza<sup>5</sup>, Antonio Torroni<sup>2</sup>  
and Paolo Ajmone-Marsan<sup>1,\*</sup>



- Turkey is one of the domestication region for goats. However, up to date these breeds have not been studied comprehensively regarding to mtDNA diversity.
- Therefore, in the present study, mtDNA of the some native goats were examined to establish links between each other genetically.



"The Goat Shepherd"  
Nahum Gutman






## In the present study;

- High levels of nucleotide diversity
- High levels of haplotype diversity
- Old haplogroups (G and A)

Turkey is important point for goat domestication

- 
- Three haplogroups (**A**, **D** and **G**) have been determined among the studied breeds .
  - Haplogroups **B** and **C**, often recognizable in Asian countries, were not found in this study.
  - This can be attributable to introgression times and human migration routes...



- Cytochrome-b results indicated that Haplogroup **G** is the most ancient haplogroup among the studied breeds, however the most contributor haplogroup to domestication was Haplogroup **A**.
- And also in the present study, a novel, cost and time-effective method is introduced for the determination of Haplogroup **G**.



- Although Angora has a different phenotype, it was not separated statistically significant.

gene flow ???

- Additional analyses

(whole mtDNA, Y chromosome etc.)



Domestic goat (*C. hircus*) is thought to originate from wild goat species:

- *C. ibex*



- *C. caucasica*



- *C. aegagrus*



- *C. prisca*

- *C. falconeri*



# Consequently...

- Inbreeding and mating with culture breeds lead to vanishing of the native breeds...
- Despite their low production values, the loss of native breeds is of quite important genetically because of their special features.
- Therefore, **embryo**, **semen**, **cell culture** and **DNA storage banks** were established within the scope of TURKHAYGEN-1. And also obtained DNAs were characterized with molecular markers such as **mtDNA**

**TURKHAYGEN-1: In Vitro Conservation and Preliminary Molecular Identification of Some Turkish Domestic Animal Genetic Resources-I**

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**It is not too late!!!**

- This data showed that the studied breeds preserved their mtDNA diversities.
- It is not too late. We have still time and opportunity for preservation of our genetic resources.

further information:[www. turkhaygen.gov.tr](http://www.turkhaygen.gov.tr)

