PHENOTYPIC TRAITS, DIVERSITY LEVELS AND GENETIC RELATIONSHIPS OF CRETAN SHEEP BREEDS

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Η Ευρώπη επενδύει στις αγροτικές περιοχές
ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ
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Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης

Introduction

- Greece's sheep farming plays a pivotal role in the Greek economy ranking **third** in Europe (**18%** of the country's total agricultural income)
- Total sheep population in Greece (2022): **7,378,000**
- Sheep population in the Island of **Crete**: **2,036,000** (**27.6%** of the total population)
- Greek sheep's milk production: 883 th. tons (**29.4%** of Europe's overall production)
- Cretan sheep's milk production: 152 th. tons (**5.1%** Europe's overall production)

Importance of indigenous sheep breeds

- The diverse indigenous Greek sheep breeds reflect a long history of domestication and evolution, showing unique traits due to natural and zootechnical selection pressures.
- There is a total of 35 – 40 indigenous sheep breeds in Greece
- In Crete island there are four indigenous breeds of sheep
- Indigenous breeds are important.

Sitia sheep

Importance of indigenous sheep breeds

- Unique traits such as:

a. Disease resistance,
b. Adaptability to harsh conditions,
c. Efficiency on poor vegetation etc.

- Low input management
- Valuable genes

Sitia sheep

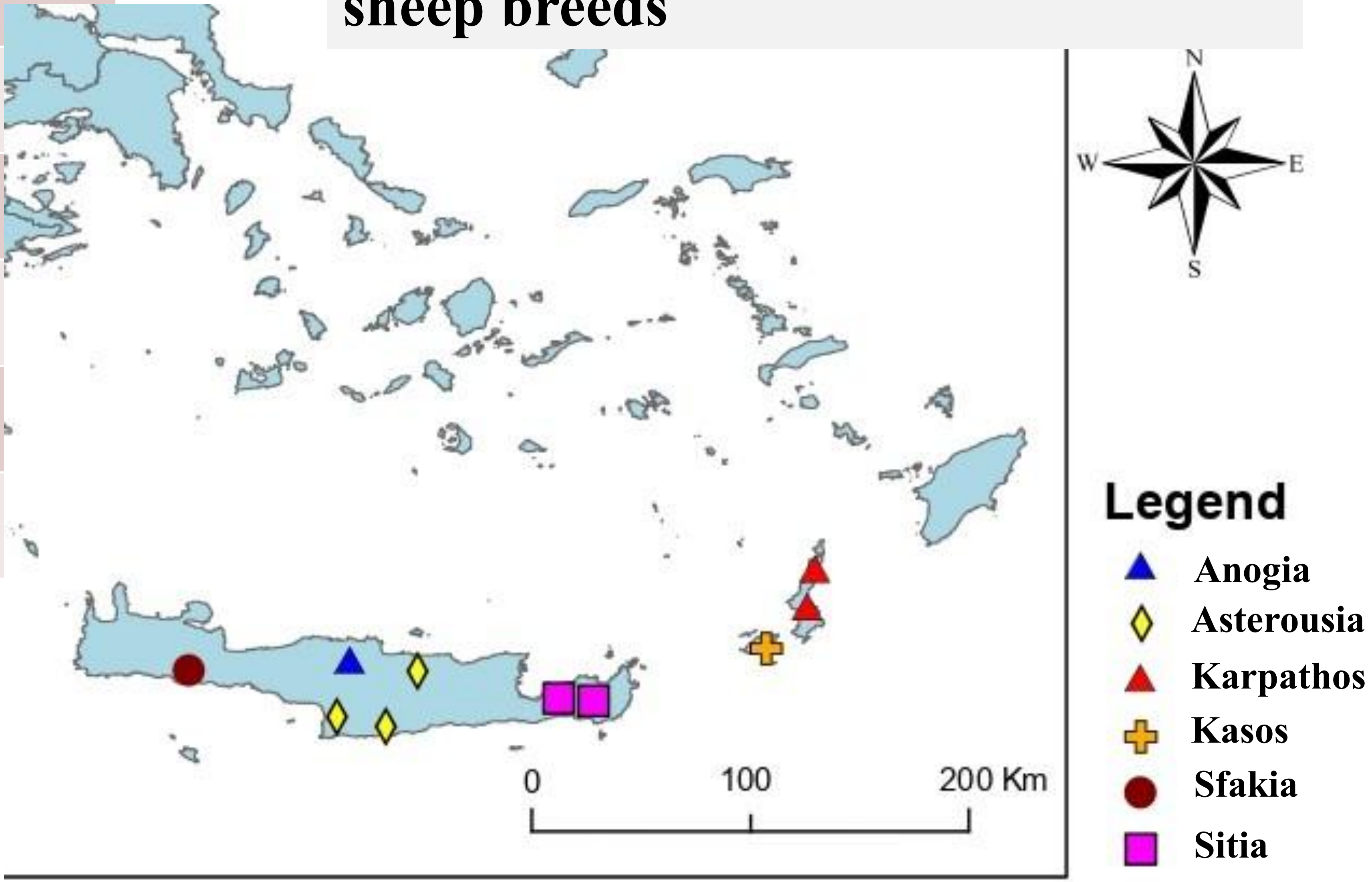
Aim of the Study

- This study aims to comprehensively define the genetic landscape of indigenous Greek Cretan sheep breeds and contribute to their protection and conservation.

Farm of Sfakia breed near Skaloti village

Materials & Methods

Breeds/ Populations	Phenotypic traits (n= 90 (♀))	Genetics (n=168)
Asterousia	23	27
Anogia	7	25
Sfakia	17	31
Sitia	43	12
Kasos	-	58
Karpathos	-	15



Sampling map of the indigenous sheep breeds

Body measurements included withers height, body length, body weight, ear length etc.

Materials & Methods: the four Cretan indigenous sheep breeds

Sfakia sheep

Asterousia sheep

Anogia sheep

Sitia sheep

Genetic analysis

Materials & Methods

- DNA was isolated (QIAamp DNA MiniKit, QIAGEN) from animal blood from **168** animals.
- OvineSNP50 BeadChip from Illumina was employed for genotyping of SNPs
- **Quality control of molecular data:**
- **45,662** marker SNPs were generated
- **4,331** multi-allelic blocks (small haplotypes of four marker SNPs) were generated.

1. Only call rate exceeding 0.95 were included,
2. SNPs that mapped to unknown or sex chromosomes were removed,
3. SNPs that were genotyped for less than 90% in all samples were excluded,
4. SNPs with a MAF <0.02 and $P \leq 0.01$ were removed.

Materials & Methods

Genetic Diversity

- Total number of alleles (n_A)
- Total number of private alleles (np_A)

Based on 4,331 multiallelic blocks (n=168):

- Observed (Ho) and expected (He) heterozygosity
- Allelic richness (AR)

Genetic relationships and inbreeding

Based on 45,662 SNP's (n=168):

- UAR table (Unified Additive Relationships)
- Runs of Homozygosity (ROH)

(Classes: 1–2 Mb, 2–8 Mb, and >8 Mb, (F_{ROH}))

Materials & Methods

RUNS OF HOMOZYGOSITY (ROH):

- a window-free approach for consecutive SNP-based detection was used. In this method, one SNP with a missing genotype and up to one heterozygous genotype was allowed within a run.
- Min ROH length was set at 1000 kb. ROH were determined for each animal and subsequently categorized into specific length classes: 1–2 Mb, 2–8 Mb, and >8 Mb
- The total number of identified ROH within these length categories was calculated for every individual in each breed. To determine the mean sum of ROH, the lengths of all ROH for every individual in the sheep populations were summed, and these totals were then averaged for each breed or population
- Genomic inbreeding coefficient based on ROH (F_{ROH}) was calculated using:

$\mathbf{F_{ROH} = \Sigma\,(L_{ROH}) / L_{GENOME}}$, ($\Sigma(L_{ROH})$: total length of all ROH per individual and L_{GENOME} : total autosomal SNP coverage (2.44 Gb)

Materials & Methods

Genetic relationships

- Proportion of shared alleles (PS) between all individuals (n=168)
- Table of genetic distances $D_{\mathbf{PS}}$, $D_{PS} = -\log(PS)$,
- Multidimensional Scaling (MDS) analysis to map the positions of the 168 individuals in two-dimensional space
- Table of Nei's genetic distances (D_A) between populations (1983),

$$D_A = 1 - \sum_{\ell} \sum_{u} \sqrt{X_u Y_u} / L$$

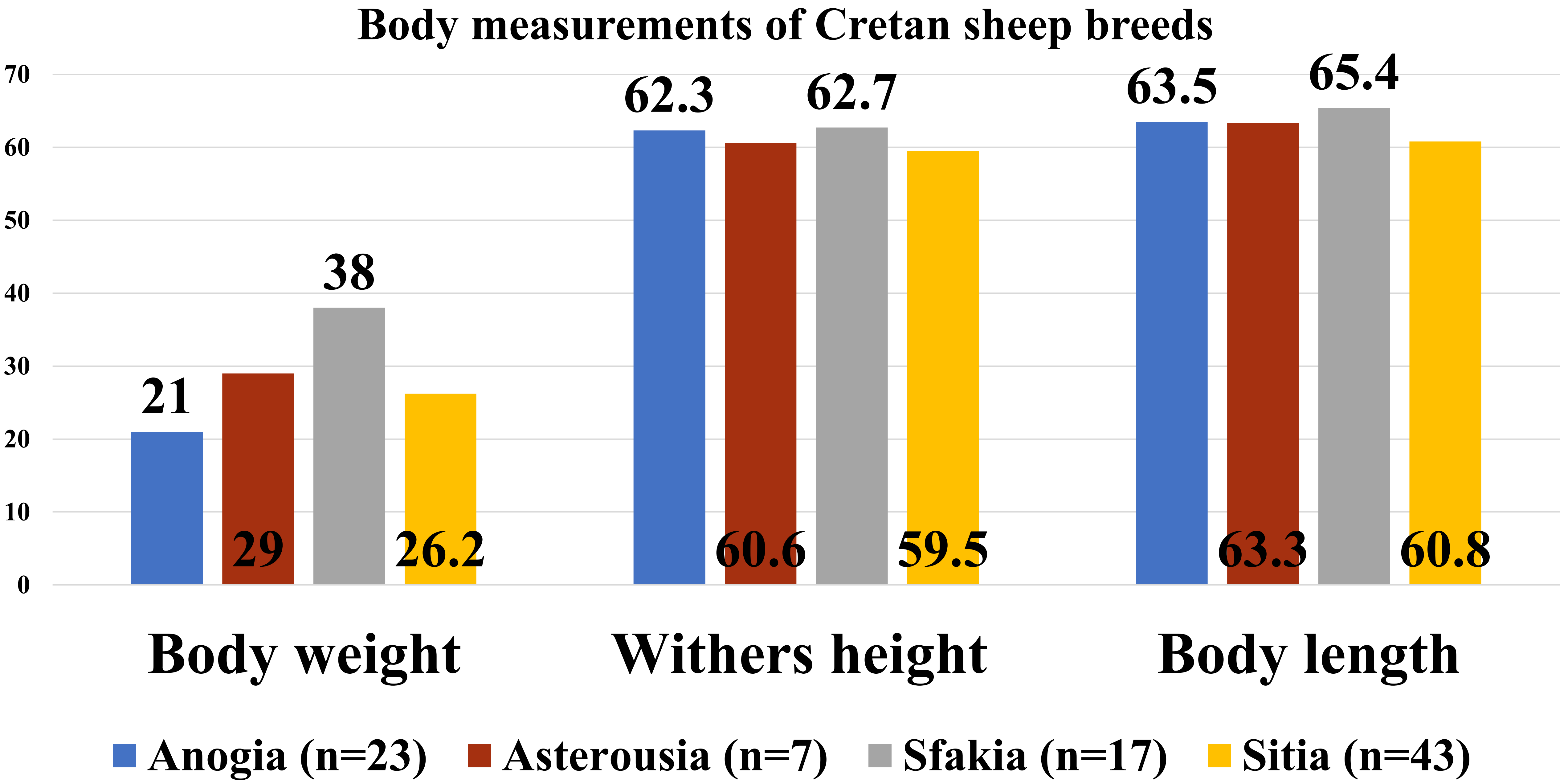
- Construction of phylogenetic trees at breed and individual levels

  (Neighbor-joining algorithm, SplitsTree4)

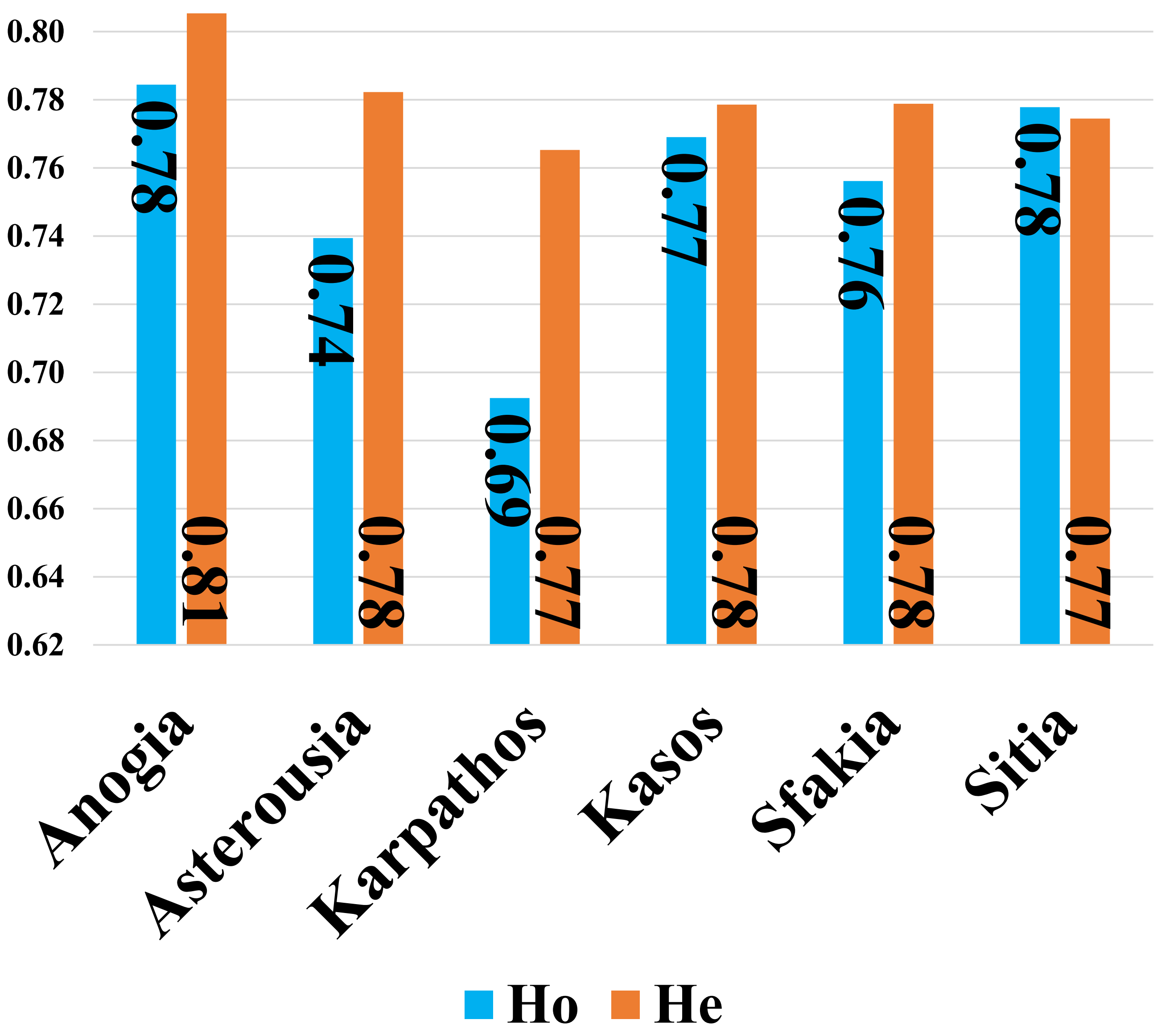
Results

Sfakia sheep

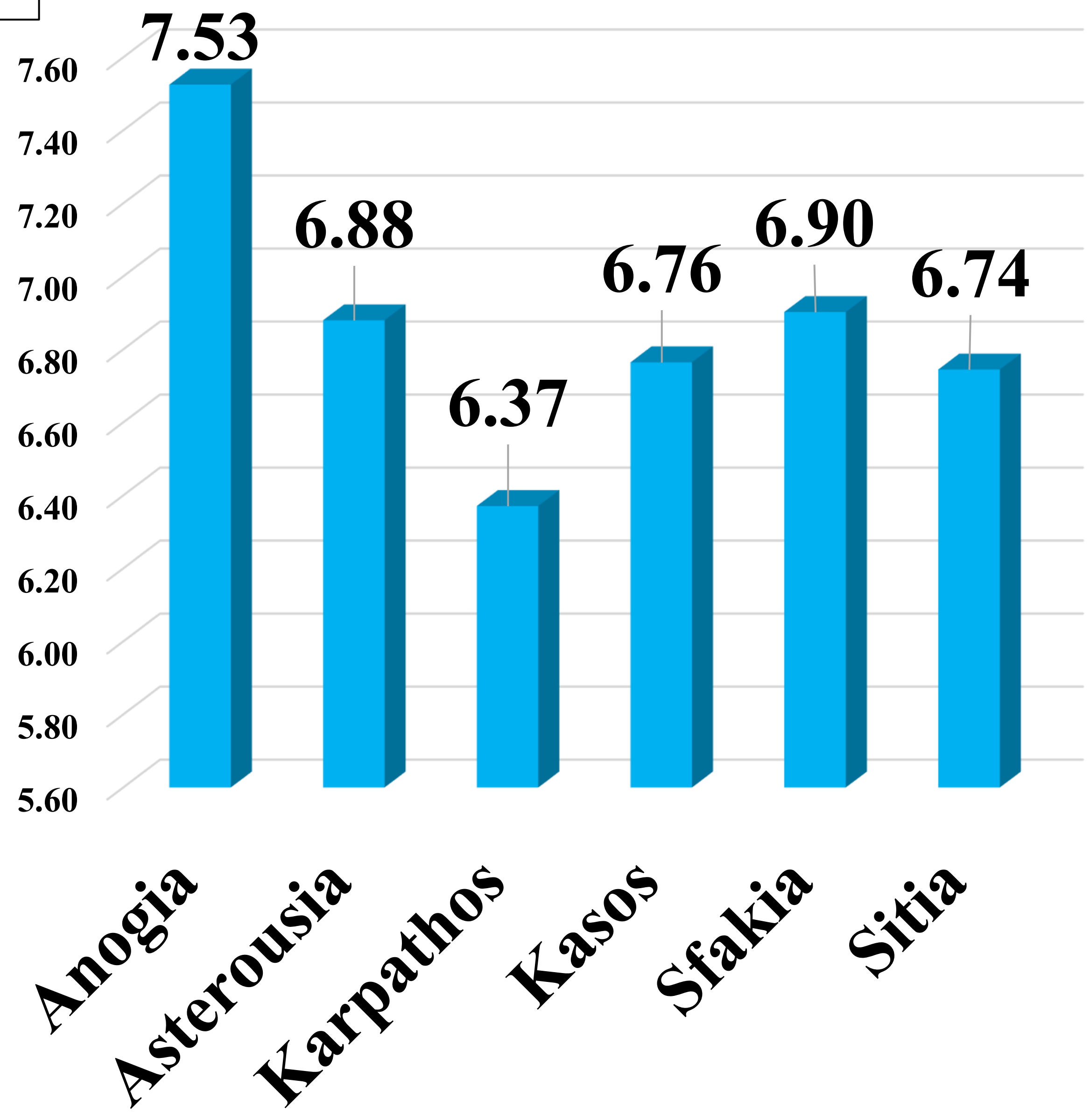
Phenotypic traits



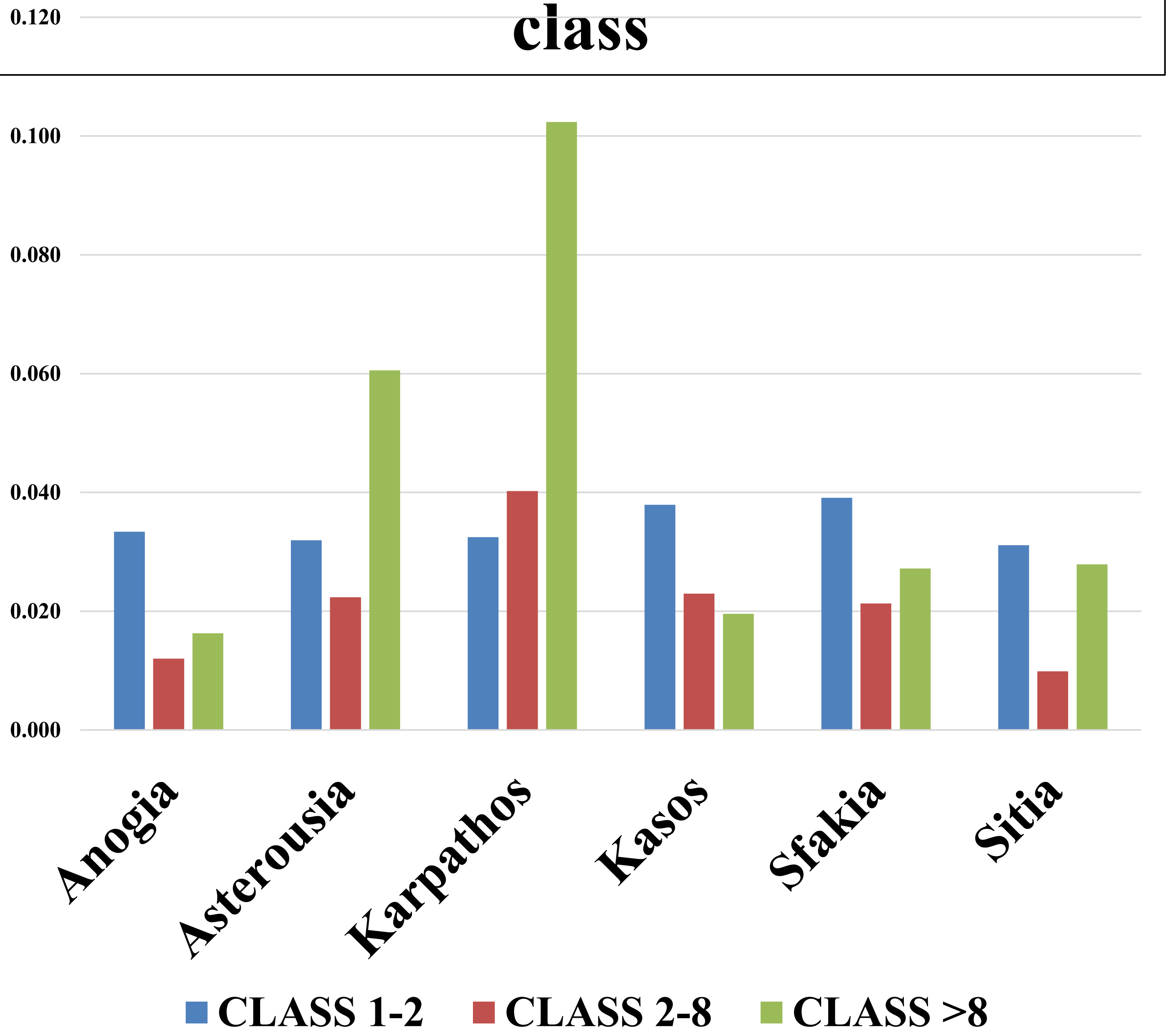
Mean observed heterozygosity (H_o) and expected heterozygosity (H_e)



Allelic richness (AR)



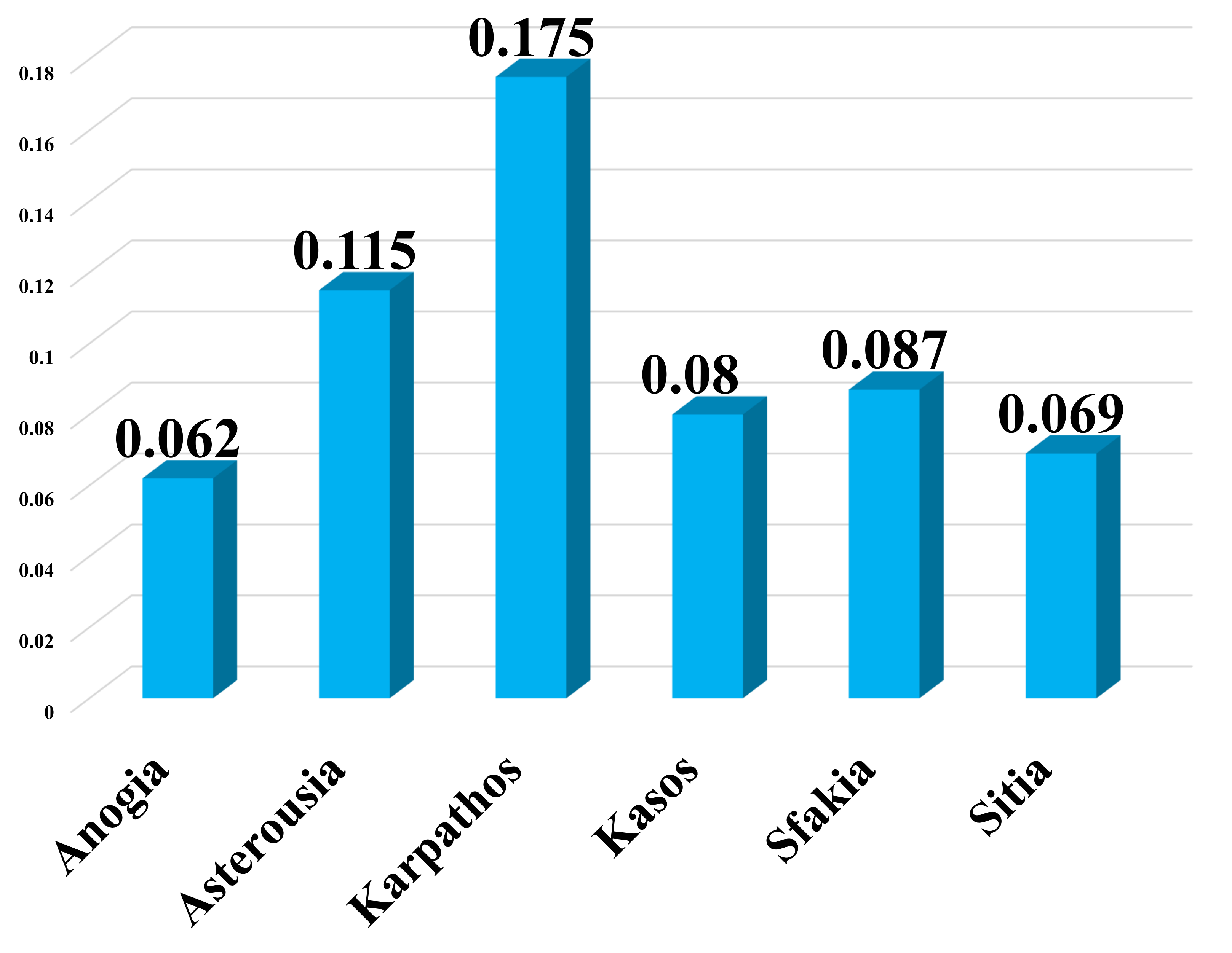
Mean inbreeding F_{ROH} per length class

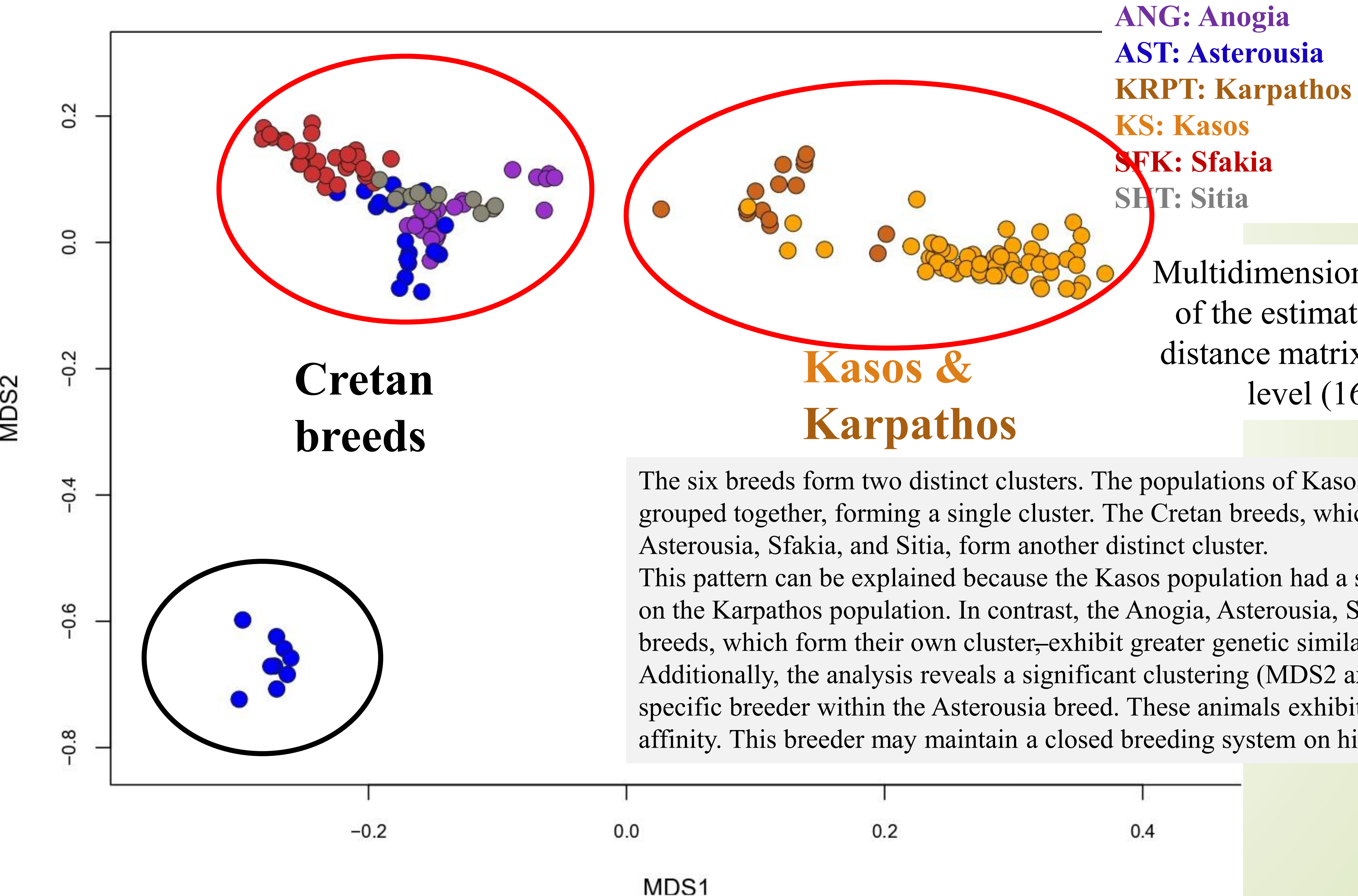


In this diagram, the mean inbreeding coefficient based on the runs of Homozygosity for each breed is presented. The left diagram presents the mean inbreeding coefficient per class. The classes are 1-2Mb with the blue colour, 2-8Mb with the red, and more than 8 Mb with the green. With these classes, we can observe the inbreeding in each breed over time, with the first one showing the oldest and the green one showing the most recent. We can see that in the past, the inbreeding in the breeds was similar. Over time, in all breeds except Kasos, inbreeding tends to increase. Especially, in the Asterousia and Karpathos breeds.

Mean inbreeding F_{ROH} (>1)

In this diagram, which presents the overall mean inbreeding coefficient, the Karpathos and Asterousia breeds show the highest genetic inbreeding coefficient. In contrast, the others have a similar lowest inbreeding coefficient. The Anogia breed has the lowest.





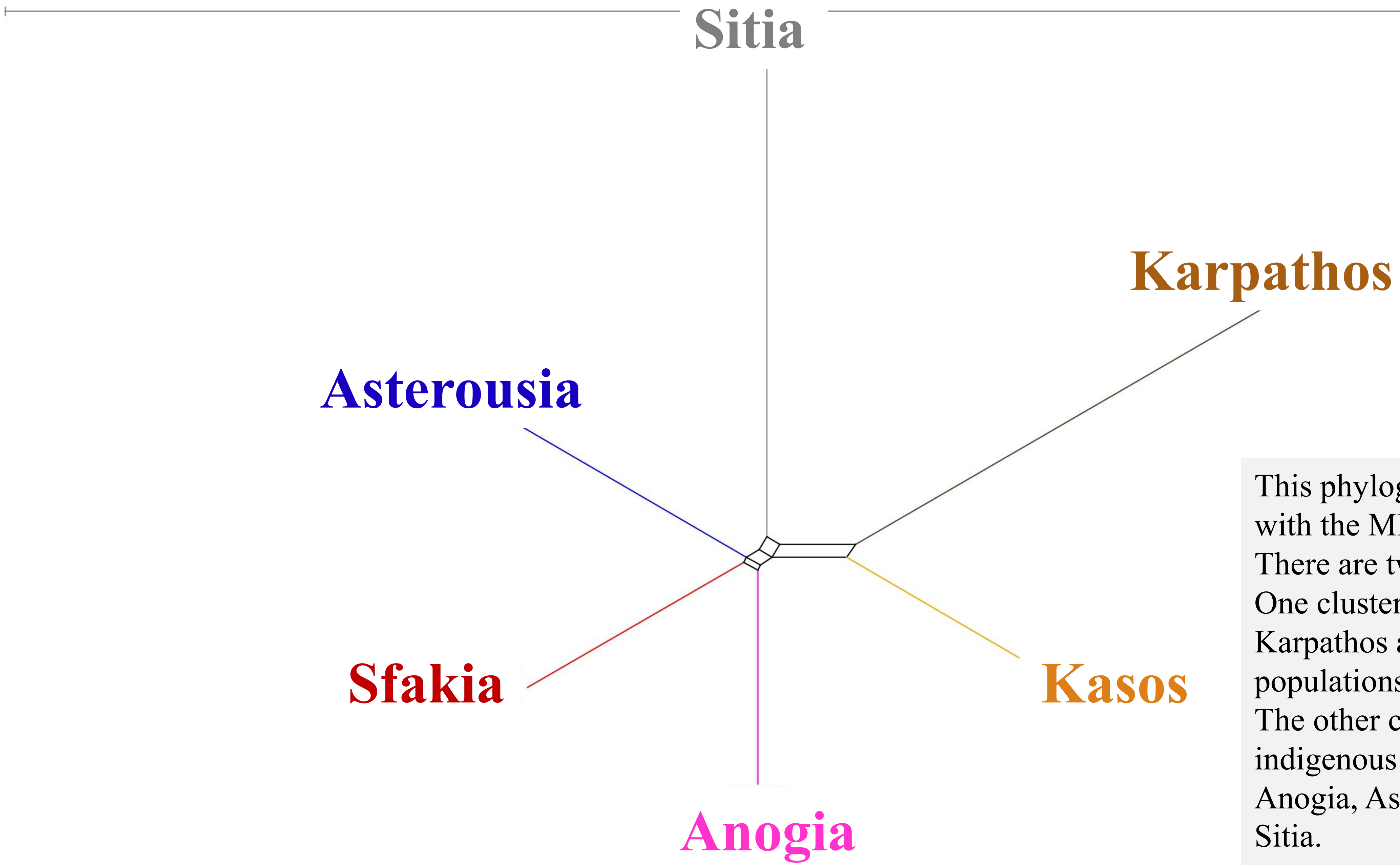
Multidimensional scaling **(MDS)** of the estimated allele sharing distance matrix at the individual level (168 animals)

The six breeds form two distinct clusters. The populations of Kasos and Karpathos are grouped together, forming a single cluster. The Cretan breeds, which include Anogia, Asterousia, Sfakia, and Sitia, form another distinct cluster.

This pattern can be explained because the Kasos population had a significant influence on the Karpathos population. In contrast, the Anogia, Asterousia, Sfakia, and Sitia breeds, which form their own cluster~~,~~ exhibit greater genetic similarity among them.

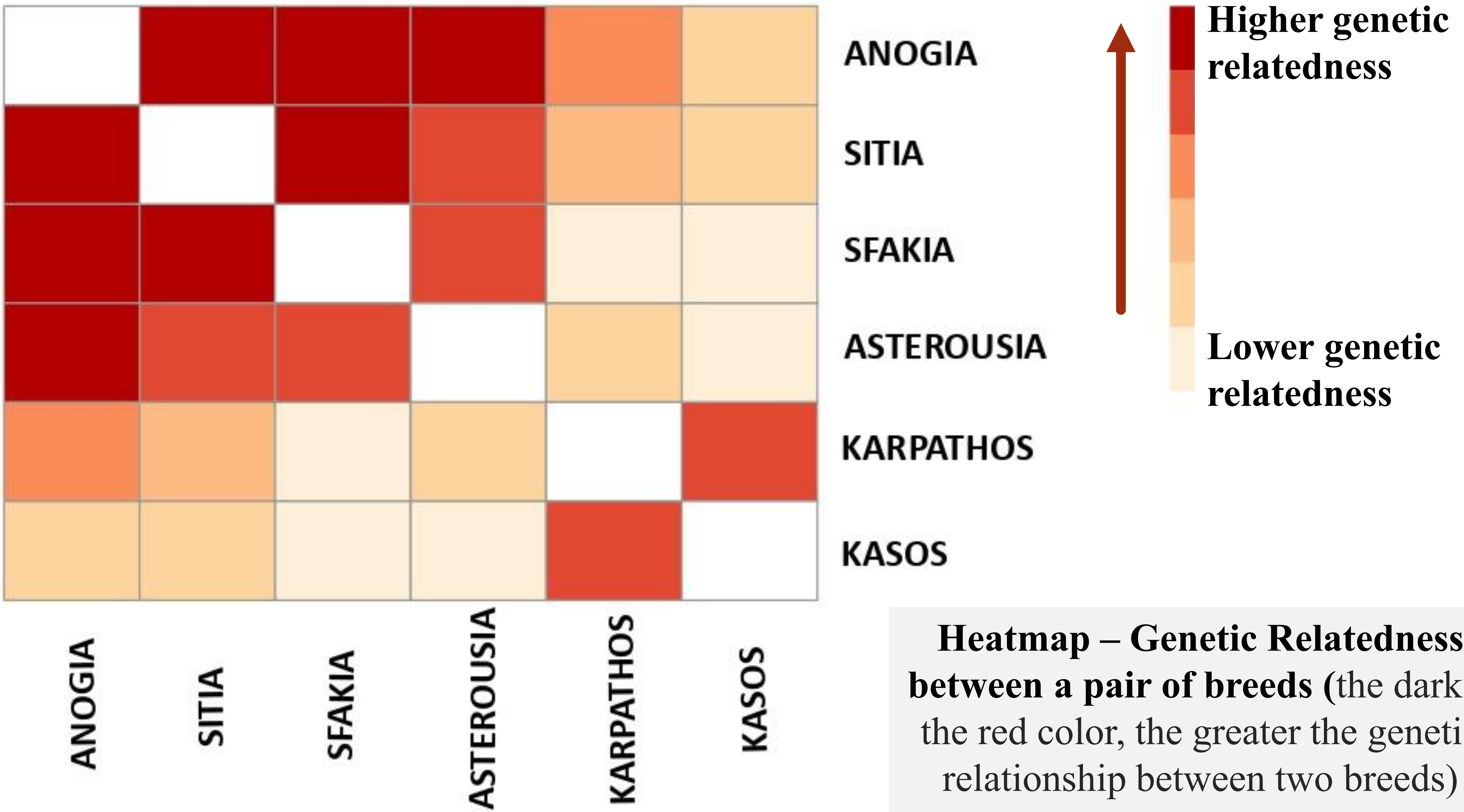
Additionally, the analysis reveals a significant clustering (MDS2 axis) of animals from a specific breeder within the Asterousia breed. These animals exhibit high levels of genetic affinity. This breeder may maintain a closed breeding system on his farm.

Neighbor-network based on pairwise Nei’s D_A genetic distances among the 6 breeds



● ANOGIA
● ASTEROUSIA
● KARPATHOS
● KASOS
● SFAKIA
● SITIA

This phylogenetic tree agrees with the MDS analysis.
There are two distinct clusters:
One cluster includes the Karpathos and Kasos populations.
The other contains the indigenous Cretan breeds: Anogia, Asterousia, Sfakia and Sitia.



Heatmap – Genetic Relatedness between a pair of breeds (the darker the red color, the greater the genetic relationship between two breeds)

Conclusions

- Cretan sheep are **small** and are consistent with their mountainous type of sheep and their extensive rearing system.
- Our findings provide the first evidence for genetic diversity and inbreeding status of indigenous sheep breeds of Crete.
- The genetic diversity parameters and genomic inbreeding coefficient indicate that the indigenous breeds are in a **critical state** in terms of their genetic background **and require our immediate attention.**
- Development of **conservation programs** aimed at preserving their **genetic diversity.**

Asterousia sheep

Thank you very much!

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