

Journal Pre-proofs

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PII: S1751-7311(26)00091-1

DOI: <https://doi.org/10.1016/j.animal.2026.101844>

Reference: ANIMAL 101844

To appear in: *Animal*

Received Date: 29 January 2026

Revised Date: 24 April 2026

Accepted Date: 24 April 2026

Please cite this article as: G. Chessari, V. Landi, S. Riggio, M.T. Sardina, E. Ciani, A. Criscione, C. Persichilli, G. Senczuk, S. Mastrangelo, Genome-wide analysis provides insights into the conservation status and the genomic architecture of Gentile di Puglia sheep, a Merino-type breed, *Animal* (2026), doi: <https://doi.org/10.1016/j.animal.2026.101844>

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Genome-wide analysis provides insights into the conservation status and the genomic architecture of Gentile di Puglia sheep, a Merino-type breed

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Highlights

Gentile di Puglia sheep show moderate to high genetic diversity and low genomic inbreeding.

Population structure reflects farm differentiation driven by breeding history and management practices.

Runs of homozygosity and heterozygosity-rich regions identify functionally relevant genes supporting conservation-oriented breeding.

Partial overlap between runs of homozygosity and heterozygosity-rich regions islands was observed.

Genomic data serves as a critical resource for informed decision-making.

Abstract

The Gentile di Puglia is a local Italian Merino-type sheep breed of high historical and economic value, known for its fine wool, and whose conservation depends on an accurate assessment of its genomic diversity. Using a medium-density Single Nucleotide Polymorphism (**SNP**) array, we analyzed the population structure, homozygosity and heterozygosity patterns of 1 337 individuals genotyped for 45 869 autosomal SNPs, representing one of the largest datasets available for a local sheep breed. Genetic diversity indices highlighted moderate to high heterozygosity and low genomic inbreeding. Population structure analysis revealed clear differentiation among farms shaped by historical breeding origins and recent management practices. Runs of homozygosity (**ROH**) analysis showed that most segments were short (< 4 Mb), indicating predominantly ancient inbreeding, likely reflecting historical demographic processes rather than recent consanguineous matings. Several recurrent ROH islands were identified on ovine chromosomes (**OAR**) 2, OAR6, OAR10, and OAR19, harboring genes associated with growth, fat metabolism, reproduction, immunity, and environmental adaptation. Heterozygosity-rich regions (**HRR**) consisted of short segments (< 1 Mb), with two HRR islands harboring genes of potential functional relevance (*VPS13B*, *CLCN3*, *NEK1*, *SH3RF1*), which may contribute to reproductive traits, disease resistance, or overall fitness. Partial overlap between ROH and HRR islands was observed, suggesting a nuanced genetic structure in which patterns of homozygosity and heterozygosity may contribute differently to shaping genetic diversity. Overall, this integrated genomic approach provides a comprehensive assessment of the current genetic status of the Gentile di Puglia breed and a solid scientific foundation for the design and implementation of effective conservation strategies. In this context, genomic data serves as a critical resource for informed decision-making.

Keywords: local sheep breed, genetic diversity, runs of homozygosity, heterozygosity-rich regions, candidate genes.

Implications

This study provides insights for conserving and managing the Gentile di Puglia, an Italian Merino-type breed. Genetic analyses indicate the population still retains considerable diversity. Genes associated with growth, reproduction, immunity, and adaptation highlight regions that could be targeted in breeding programs to improve fitness and production traits without reducing diversity. Farm-level differentiation underscores the need to consider breeder histories and maintain controlled gene flow. Integrating genomic data offers a powerful tool to monitor inbreeding, guide

management, and safeguard the long-term viability of this historically and economically important breed.

Introduction

The Gentile di Puglia, also known as the Apulian Merino, is a local Italian sheep breed renowned for its fine wool quality. It is one of the few autochthonous Merino-type breeds reared in Italy, and represents a valuable genetic resource within the national livestock heritage (Santillo et al., 2025). Historical records mention that, during the 15th century, Merino rams were imported from Aragon (Spain) to Southern Italy, where they were mated to native ewes, giving rise to the contemporary Gentile di Puglia breed (Sarti et al., 2006). Subsequent genetic analyses suggest that this breed has preserved traces of its original Merino ancestry, despite historical gene flow from other Mediterranean and European sheep populations (Ciani et al., 2015). Over time, production objectives shifted towards meat and milk, although recent interest in sustainable and locally sourced materials has renewed attention to its wool production. Like many Italian local breeds, the Gentile di Puglia has experienced a marked demographic decline in recent decades. According to the Italian National Livestock Register (June 2025), the population comprises 9 505 individuals distributed across approximately 40 farms (<https://www.vetinfo.it/>). However, only a subset of this population, approximately 4 000 animals from 16 farms, is actively enrolled in the herdbook and participates in the official breeding and selection program managed by the Italian Sheep and Goat Breeders Association (**Asso.Na.Pa**) (<https://www.assonapa.it/>). Within the registered population, males account for approximately 7% and females for 93%. This breed is classified as vulnerable by the Domestic Animal Diversity Information System (**DAD-IS**) of FAO (<https://www.fao.org/dad-is/browse-by-country-and-species/en/>). Previous studies have highlighted both its excellent wool quality, characterized by ultrafine and fine fiber classes and good fleece homogeneity, and its distinctive genetic background, emphasizing the importance of conservation programs to safeguard this breed from further genetic erosion (Moioli et al., 2006; Sarti et al., 2006; d'Angelo et al., 2009; Ciani et al., 2013).

The availability of ovine genome-wide Single Nucleotide Polymorphism (**SNP**) panels has provided unprecedented insights into the genome structure of both local and cosmopolitan sheep breeds (Kijas et al., 2012; Mastrangelo et al., 2018a; Deniskova et al., 2021; Chessari et al., 2023b). A key advantage of SNP-based approaches is the possibility to identify Runs of Homozygosity (**ROH**), continuous homozygous segments inherited identically by descent. ROH provide a direct genomic measure of autozygosity and are informative for distinguishing between recent and ancient inbreeding events, as well as reconstructing past demographic processes (McQuillan et al., 2008; Peripolli et al., 2017). Complementary to ROH, the identification of heterozygosity-rich regions (**HRR**) provides further insight into genomic areas under balancing selection, introgression, or reduced autozygosity. Together, ROH and HRR analyses offer a comprehensive view of genomic structure and diversity, supporting the development of effective conservation and breeding strategies (Selli et al., 2021; Chessari et al., 2024; Biscarini et al., 2026). Therefore, genomic tools are essential

for accurately quantifying current levels of genetic diversity and for assessing potential vulnerabilities associated with demographic history and management practices in local breeds.

In this study, we used a medium-density SNP array (50 K) to investigate the genomic architecture and population structure of the Gentile di Puglia sheep breed. Our specific objectives were to assess levels and distribution of autozygosity through ROH, to identify HRR, and to characterize patterns of genetic diversity and population structure within the breed. By integrating these genomic approaches, the study aims to provide a detailed characterization of the breed's genetic status and to generate information useful for future conservation and breeding strategies.

Material and Methods

Sampling, genotyping and quality control

A total of 2 035 adult Gentile di Puglia (**GDP**) sheep (79 males and 1956 females) were sampled from seven farms located in Southern Italy (Supplementary Table S1 and Supplementary Figure S1). The seven farms included in this study were selected among those participating in the official breeding program and registered in the herdbook, prioritizing those with the largest number of animals. These farms collectively account for more than 90% of the registered animals in the country.

DNA was extracted from blood samples using the commercial Illustra Blood Genomic Prep Mini Spin kit (GE Healthcare, Little Chalfont, UK). The samples were genotyped using the Illumina Ovine SNP50K BeadChip array, which includes 64 734 raw SNPs spanning the entire ovine genome (Illumina, San Diego, California, USA). Lifter of genome assembly was performed for genomic coordinates and *rs* IDs using PLINK v1.9 (Chang et al., 2015) and the latest Ensembl release 115 (accessed on 10 September 2025) for the ARS-UI_Ramb_v3.0 (GCA_016772045.2). PLINK v1.9 (Chang et al., 2015) was used to exclude unmapped SNPs and markers on sex chromosomes. To minimize the inclusion of closely related individuals, a relatedness filter was applied using pairwise identity-by-descent (**IBD**) estimates computed in PLINK. Pairs of individuals with **PI_HAT** values above 0.4 were identified, and one individual from each pair was removed to reduce the impact of close kinship on subsequent analyses. Moreover, quality filtering was performed, setting the marker missing call rate to 0.05, the minor allele frequency to 0.01, and the individual genotype missingness to 0.10.

Genetic diversity statistics

PLINK v1.9 (Chang et al., 2015) was used to estimate genetic diversity coefficients, including observed (H_O) and expected heterozygosity (H_E), molecular inbreeding coefficient (F_{IT}), and the minor allele frequency (**MAF**). Moreover, the squared correlation coefficient of allele frequencies at pairs of loci (r^2) was used as a measure of linkage disequilibrium (**LD**). Pairwise r^2 values were estimated for all SNP pairs separated by up to 1 000 kb (McKay et al., 2007). To summarize LD decay, pairwise r^2 values were grouped into 10-kb distance bins, the mean r^2 within each bin was calculated, and the LD decay curve was plotted using *ggplot2* package (Wickham, 2016) in R environment (RCoreTeam, 2025). The effective population size (N_e) was

also calculated with the SNeP software using default settings (Barbato et al., 2015). In addition, individual inbreeding coefficients (F_{IS}) were estimated for all animals, and their distributions were then compared across the seven sampled farms. These values were visualized as violin plots in R (RCoreTeam, 2025) using the *ggplot2* package (Wickham, 2016), to provide a clear representation of variability in inbreeding among farms.

Population structure between farms

Genetic relationships among the seven farms were assessed using a pruned dataset. Markers in high LD were removed using the *indep-pairwise 50 5 0.2* function in PLINK v1.9 (Chang et al., 2015), thus resulting in 35 258 SNPs. Multidimensional scaling (**MDS**) analysis on pairwise identity-by-state (**IBS**) distances between individuals was performed. The graphical representation of the MDS results was generated in the R environment (RCoreTeam, 2025), to capture the patterns of genetic variation among individuals. A network-based analysis was performed to investigate fine-scale population structure. Network construction and individuals' clustering were implemented using the R version of NetView v2.1.0.9000 (Neuditschko et al., 2012; Steinig et al., 2016). The method requires specification of the number of k-nearest neighbours (**K-NN**) retained for each individual. K-NN was set up from 10 to 80, to investigate the characteristics of both fine- and large-scale genetic structures. Sub-population differentiation was quantified using pairwise population differentiation index (F_{ST}) and Reynolds distances estimated with Arlequin v3.5.2.2 (Excoffier and Lischer, 2010), and then visualized as a heatmap using the R package *ggplot2* (Wickham, 2016). Additionally, a dendrogram was generated from the F_{ST} distances using the average linkage method implemented in the hierarchical clustering (**UPGMA**) function *hclust* of the standard R package *stats* (RCoreTeam, 2025). Finally, ancestry proportions were estimated using the unsupervised model-based clustering algorithm implemented in Admixture v1.3.0 (Alexander et al., 2009), with the most likely number of ancestral populations (**K**) determined by cross-validation errors across *K* values. The inferred ancestral Q matrix was visualized using the R package *BITE* v1.2.0008 (Milanesi et al., 2017).

Runs of homozygosity

ROH were investigated using the sliding windows method implemented in *detectRUNS* R package (Biscarini et al., 2018), by setting a minimum ROH length of 1 Mb with a maximum gap of 250 kb between two consecutive SNPs, a window size of 25 SNPs with a minimum number of 20 SNPs, a threshold of 0.05 and a density of one SNP every 100 kb, excluding missing or opposite SNPs both in a run and in a window. ROH results were classified in five different length classes (Ferenčaković et al., 2013) and summarized into statistical indices, such as the mean number of ROH per individual (N_{ROH}), the average length of ROH in Mb per individual (L_{ROH}), the total length covered by ROH segments in Mb (S_{ROH}) and the population genomic inbreeding coefficient (F_{ROH}) calculated as the ratio between S_{ROH} and the total autosomal genome length covered by SNPs (approx. 2.46 Gb for sheep).

In addition, inbreeding coefficients were calculated per chromosome using all individuals for which ROH estimates were available. The frequency of SNP occurrence within ROH was calculated across all individuals. SNPs falling within the

top 0.5% of the empirical distribution were defined as constituting ROH islands. Genes and quantitative trait loci (**QTLs**) overlapping with these SNPs were annotated using Ensembl's Variant Effect Predictor (**VEP**) (McLaren et al., 2016).

Heterozygosity rich regions

HRR were investigated using similar parameters previously implemented by Chessari et al. (2024), following the sliding windows method: no missing or opposite genotypes were allowed in a run nor in a sliding window, the maximum gap between consecutive SNPs was set to 1 Mb, while the minimum HRR length to 250 kb; the window size was set to 10 SNPs considering a minimum number of 10 markers, a threshold of 0.05 and a density of one SNP every 100 kb. Similar to ROH detection, HRR were classified into three length classes (0.25 Mb - 0.50 Mb, 0.50 Mb - 1.00 Mb, > 1.00 Mb) and summarized with corresponding statistical indices: the mean number of HRR per individual (N_{HRR}), the average length of HRR in Mb per individual (L_{HRR}), the total length covered by HRR segments in Mb (S_{HRR}) and the population genomic diversity coefficient (D_{HRR}) (Bordonaro et al., 2023). SNPs within the top 0.1% of the markers' percentage distribution were considered to define HRR islands, for which gene and QTL annotations were performed as for ROH islands.

Results

The final filtered dataset comprised a total of 1 337 individuals genotyped for 45 869 high-quality SNPs mapped on ovine autosome chromosome (**OAR1-OAR26**) (Supplementary Figure S2), where the marker density expressed as SNPs per Mb was 18.68, while the average spacing between consecutive markers was 53.53 kb. The reduction in sample size was mainly due to the removal of closely related individuals.

Genetic diversity statistics

All genetic indices' results are summarized in Table 1. The breed showed a moderate level of genetic variability, with H_O and H_E values close to each other (0.374 ± 0.118 and 0.383 ± 0.120 , respectively), also confirmed by the relatively low average of F_{IT} (0.022 ± 0.036). Inbreeding showed a shared trend across herds (Supplementary Table S2), with negative mean F_{IS} values; nevertheless, large standard deviations revealed substantial variability in individual inbreeding levels within each herd. Similarly, the distribution of individual F_{IS} values is similar among farms, with slight excesses of homozygosity in F6 and F7, and some individuals showing higher heterozygosity in F2 and F7 (Supplementary Figure S3).

Levels of pairwise LD decreased with increasing physical distance between SNPs (Supplementary Figure S4A), indicating a consistent LD decay. Mean r^2 values, averaged within 10-kb distance bins, dropped below 0.05 at approximately 100 kb, reflecting moderate genome-wide recombination and variability, while the mean r^2 calculated from all pairwise SNP comparisons pooled over all autosomes was 0.029. A progressive decrease in N_e over generations was observed, with estimates indicating an N_e of approximately 623 about 13 generations ago (Supplementary Figure S4B).

Population structure among farms

Genetic relationships among farms were assessed using pairwise distances (Fig. 1). Scatterplots of IBS distances are illustrated in Fig. 1A. Components C1 (13.84%) and C2 (11.65%) explained most of the variance. Along C2, F2, F3, and F6 overlapped, while F5 and F7 were clearly separated. F4 formed a compact cluster, and F1 was spread across both C1 and C2 axes, positioned between F5, F7, and the central cluster. Supplementary Figures S5A and Supplementary Figures S5B showed clear separation among all farms, with some evidence of introgression: F4 and F1 clustered with F6, while some F7 animals grouped within F5. Network visualization analysis provided a fine-tuned resolution of connectedness within and between farms. At a K-NN value of 10, there exists a massive interconnection of networks among individuals (Supplementary Figure S6A). This, however, excluded animals from F7 that formed a separate cluster. The farm clusters were evident starting with K-NN =20 (Supplementary Figure S6B). More closely related individuals are co-located and only few individuals from F3 remained unassigned. Using an intermediate number of nearest neighbors (K-NN = 36), animals clustered into groups and networks that corresponded to their farm or geographic origin (Fig 1B). These groups are consistently retained up to k-NN =80 (Supplementary Figure S6C). Pairwise F_{ST} and Reynolds distances were plotted as heatmaps (Fig. 1C) and show average genetic relatedness. F7 was the most distinct from the other groups (overall mean ~ 0.051), while F1 and F2 showed the closest relatedness with other farms (maximum value with F6 and F3). The dendrogram (Fig. 1D) confirmed these patterns, with F1 clustering with F5, F2 with F3, and F7 being the most genetically distinct. Finally, the Admixture analysis (Supplementary Figure S7) revealed that at $K = 2$, F7 and F2/F3 formed two distinct homogeneous groups, whereas all other farms showed mixed patterns. At $K = 7$, each farm generally exhibited a distinct profile, with F1 and F3 highlighting the more admixed genetic patterns.

Runs of homozygosity

Across the dataset, a total of 31 342 ROH were identified in 1 335 out of 1 337 animals, with an average of 23.48 ROH per individual and a maximum of 114 (Supplementary Figure S8A). Summary statistics for all ROH metrics are reported in Table 2. The mean ROH length was 3.51 Mb, with a clear predominance of short segments. Indeed, the most frequent ROH length class consisted of segments shorter than 2 Mb (38.03% of all ROH), followed by the 2-4 Mb class (30.81%), while the most affected chromosome was OAR1 (Fig. 2A). The mean S_{ROH} across individuals was 92.33 ± 72.55 Mb, with values ranging widely from 1.02 Mb to 521.35 Mb. The number of ROH per individual was strongly correlated with the total genomic length in ROH (Pearson's $r = 0.92$, p -value < 0.001) (Supplementary Figure S8B), indicating that individuals with more ROH also tend to accumulate longer cumulative ROH segments. The individual with the highest ROH count (114) exhibited an S_{ROH} of 271.72 Mb, whereas the three individuals with the highest S_{ROH} values (521.35 Mb, 434.68 Mb, and 400.64 Mb, corresponding to more than 17% of the total genome length) carried 84, 57, and 57 ROH, respectively. This pattern highlights that some individuals are characterized not only by numerous ROH but also by particularly long segments. Genome-wide F_{ROH} values ranged from 0.0004 to 0.211, with a mean of 0.037 ± 0.038 (Table 2). In contrast, chromosome-specific F_{ROH} values averaged 0.091 (Supplementary Table S3), reflecting the proportional coverage of each

chromosome by ROH rather than the absolute number of ROH segments (Fig. 2A). While the longest chromosomes (OAR1, OAR2 and OAR3) harbored the highest number and total length of ROH, their ROH-based inbreeding coefficients were comparatively lower due to their larger genomic size (~0.048). Conversely, shorter chromosomes exhibited higher F_{ROH} values (> 0.100), indicating a greater relative contribution of ROH to chromosome-specific inbreeding (Supplementary Table S3).

The top 0.5% of the percentile distribution of SNP recurrence within ROH corresponded to a threshold of 8.68% (i.e., SNPs present in at least 116 individuals), resulting in the identification of 239 markers, as shown in the Manhattan plot in Fig. 3A. The distribution of these SNPs revealed clear high-recurrence peaks on OAR2, OAR6, OAR9, OAR10 and OAR19, delineating a total of eight ROH islands in the breed (Table 3). The genomic coordinates of the ROH islands varied in size, ranging from short intervals of approximately 0.87 Mb to broader regions spanning up to ~5.00 Mb. The number of SNPs per island ranged from a minimum of 12 to a maximum of 54, with OAR19 containing the most prominent hotspot, where the maximum recurrence reached nearly 35%. These regions collectively harbored 32 genes and seven annotated QTLs (Table 3).

Heterozygosity rich regions

HRR analysis identified a total of 12 315 segments across 1 336 individuals (Table 2). On average, 9.22 HRR per individual were detected, with a relatively low standard deviation (3.07) and a maximum of 22 segments (Supplementary Figure S8A). OAR2 showed the highest number of HRR (Fig. 2B), most of which were shorter than 1 Mb. Accordingly, 98.90% of the total HRR belonged to the short length classes 0.25–0.50 Mb (53.69%) and 0.50–1.00 Mb (45.21%) (Table 2). Similarly to what was observed for ROH, a strong and highly significant correlation was detected between N_{HRR} and S_{HRR} (Pearson's $r = 0.96$, p -value < 0.001) (Supplementary Figure S8C). The mean S_{HRR} across individuals was 4.79 ± 1.66 Mb, with relatively limited maximum values (up to 11.96 Mb), suggesting moderate inter-individual variability. The mean L_{HRR} was 0.52 Mb across individuals, indicating that HRR are predominantly short and fragmented, consistent with localized patterns of genomic heterozygosity rather than extended heterozygous tracts (no runs > 2 Mb). Genome-wide D_{HRR} values ranged from 0.0004 to 0.0049, with mean and median values close to 0.002, indicating a relatively uniform distribution with minimal skewness. Shorter chromosomes showed higher D_{HRR} (> 0.010), whereas the three longest chromosomes displayed consistently low values (0.003-0.004) (Supplementary Table S3).

The identification of HRR islands was based on the top 0.1% of SNPs recurring within heterozygosity-rich regions. The Manhattan plot shown in Fig. 3B revealed peaks on four chromosomes (OAR2, OAR3, OAR9, and OAR10), corresponding to 5 HRR hotspots (Table 3). The 0.1% threshold corresponded to a minimum SNP recurrence rate of 11.89%, that is, SNPs shared by at least 159 individuals, with a maximum observed recurrence of 20.80%. Overall, 47 SNPs were included within these HRR islands. The annotated regions harbored four genes (*CLCN3*, *NEK1*, *SH3RF1*, and *VPS13B*) and three QTLs (Table 3).

Discussion

This study represents one of the most extensive genomic investigations conducted to date on the Gentile di Puglia sheep. A total of 2 035 animals were sampled from seven farms, ensuring wide geographic and genetic variability across the breed's distribution area. Notably, the filtered dataset used in this study covers approximately 21% of officially registered Gentile di Puglia sheep, highlighting the representativeness of the sampled population and strengthening the reliability of the genomic inferences drawn from our analyses.

The marker density obtained after quality control provides an adequate genome-wide resolution for the reliable assessment of within-breed variability (Kriaridou et al., 2023; Falchi et al., 2024). Previous studies on this breed have predominantly used microsatellites (Moioli et al., 2006; d'Angelo et al., 2009) and SNP markers (Ciani et al., 2014; Mastrangelo et al., 2018a; Chessari et al., 2023a), typically considering subsamples of fewer than 80 individuals. Biodiversity-oriented analyses conducted within a single sheep breed rarely achieve comparably large sample sizes (Ramljak et al., 2024; Falchi et al., 2025). Thus, large within-breed datasets substantially improve the power to detect subtle population sub-structure and fine-scale genetic patterns, thereby supporting the development of more informed and sustainable mating strategies both within and among farms (Hampton et al., 2019).

Genetic diversity statistics

Regarding genetic diversity indices, Ciani et al. (2015) reported $H_E = 0.351$ and inbreeding = -0.036 in a subset of 24 individuals; in general, our results fall within the range reported in previous studies focused on Italian local breeds (Ciani et al., 2014; Mastrangelo et al., 2014). The average MAF (0.296 ± 0.130) indicates a moderately balanced distribution of allelic frequencies across the genome. This value, as for other cosmopolitan and local sheep breeds, reflects a satisfactory level of genetic diversity within the population (Gurgul et al., 2021; Han et al., 2024). Despite methodological and sampling differences, previous studies consistently reported relatively high genetic variability for Gentile di Puglia, with low molecular coancestry (Moioli et al., 2006; d'Angelo et al., 2009).

Considering the effective population size and a generation interval of $\sim 2/3$ years in sheep (Rafter et al., 2022), the recent N_e (13 generations ago) estimated here (623) indicates a population that is not critically small and retains a moderate reservoir of genetic variation. This estimate is broadly consistent with N_e values reported for several Italian and Mediterranean local breeds (Kijas et al., 2012; Ciani et al., 2014; Baazaoui et al., 2021; Giovannini et al., 2024), although lower values have been observed in some endangered populations. Overall, the recent N_e of the Gentile di Puglia indicates a population size that, while larger than that of critically endangered local breeds, remains sufficiently small to warrant continued monitoring and management, such as promoting rams exchange and avoiding mating among close relatives, to limit further increases in inbreeding. If we convert recent N_e to an approximate census size (N_c) using commonly observed N_e/N_c ratios, which typically range between ~ 0.1 and ~ 0.3 depending on species and management (Hoban et al., 2021), the corresponding census size would be on the order of a few thousand individuals (roughly 2 000-6 000 under conservative assumptions). This rough approximation should be interpreted with caution because N_e/N_c ratios vary widely and depend on mating system, variance in reproductive success, and

management practices (Hoban et al., 2021), but it is still consistent with the documented N_c (9 505 individuals - <https://www.vetinfo.it/> - June 2025). Moreover, the observed LD decay in Gentile di Puglia is reliable with the estimated recent N_e ; the relatively rapid decline of LD reflects a moderately large effective population (Salehi et al., 2025), allowing sufficient recombination to break down allele associations over short genomic distances. However, similar LD patterns may also arise from historical or recent introgression events involving genetically differentiated stocks.

Analyses of farms relationships

The analysis of genetic variation and relationships in the Gentile di Puglia breed across the seven farms revealed distinct patterns shaped by both historical breeding origins and recent management practices, with individual farmer decisions providing essential context for interpretation. The F2 nucleus, historically a major source of sires, shows strong genetic influence across multiple groups (Ciani et al., 2013). In contrast, the F7 nucleus represents a largely isolated lineage with minimal recent introgression. Its distinct positioning in both in MDS and admixture analyses, together with its basal placement in the phylogenetic tree, suggests that F7 retains a relatively ancestral genetic composition. Other nuclei, such as F6 and F1, display mixed origins, reflecting genetic contributions from F2, F3, and recently introduced sires. These patterns are visible in their intermediate MDS positions and blended admixture profiles. The F5 nucleus, representing an ancient population from Molise, a region neighboring Apulia, shows little evidence of recent gene flow and forms a compact, differentiated cluster consistent with relatively prolonged isolation. Conversely, F4, originally part of the Molise lineage, shows evidence of introgression from F2, an inference supported by both ancillary information on recent farm management and the observed genomic patterns. Deeper understanding of the connections within and among breeds was provided by the NetView results, which corroborated the ADMIXTURE and MDS findings. In the current study, we used different values for looking both at small and large-scale structures. Within the Gentile di Puglia breed, interconnectedness between genotypes of different flocks may imply a breeding management system where animals, especially rams, are either exchanged or sold between breeders. The deliberate exchange of animals between different flocks in an organized mating scheme can be beneficial in restricting inbreeding rates.

Overall, the results confirm the high genetic variability of the breed (Moioli et al., 2006; d'Angelo et al., 2009; Ciani et al., 2013) highlighting the conservation value of isolated nuclei such as F7 and F5, which retain unique genetic signatures. In contrast, nuclei with mixed origins reflect historical and recent gene flow, contributing to population viability by reducing inbreeding risk. These results underscore the need for balanced conservation strategies that protect genetically distinct nuclei while maintaining controlled gene flow to ensure long-term breed sustainability.

Runs of homozygosity and heterozygosity-rich regions

To date, only a limited number of studies have investigated ROH in such a large number of samples (Purfield et al., 2017; Signer-Hasler et al., 2019; Bakoev et al., 2025). The importance of large sample sizes in this type of analysis lies in the ability to identify recurrent ROH across multiple individuals, thereby highlighting genomic

regions characterized by high homozygosity, potentially shaped by selection or linked to economically important traits (Weigel, 2001; Mastrangelo et al., 2018b; Nani and Penagaricano, 2020). Moreover, meaningful comparisons can still be made across studies because many sheep breeds have been genotyped using the same BeadChip and similar sliding-window detection approaches with comparable parameter settings, making results directly comparable (Abdoli et al., 2023; Chessari et al., 2023b; Giovannini et al., 2024; Szmatoła et al., 2025).

The well-established relationship between ROH segment length and the temporal proximity of inbreeding events (Eusebi et al., 2019) is clearly reflected in our results: approximately 69% of ROH segments are shorter than 4 Mb, indicating that inbreeding in the Gentile di Puglia breed is predominantly ancient. This interpretation is supported by the low mean F_{ROH} values and the relatively high levels of observed heterozygosity (H_O) suggesting that although selection has effectively shaped the desired phenotypic traits (Sarti et al., 2006), it has not resulted in excessive inbreeding within the breed. Moreover, the predominance of short ROH segments may also reflect historical admixture and gene flow with other populations, which can disrupt long homozygous tracts and contribute to the maintenance of genetic diversity (Ceballos et al., 2018). At the same time, our results indicate a heterogeneous inbreeding pattern at the individual level. While some animals lacked long ROH segments, others exhibited extended homozygous tracts, suggesting the occurrence of both ancient and more recent inbreeding events within the population. Consequently, certain individuals carry long ROH segments more frequently than would be expected in large, genetically diverse populations. From a conservation and breeding perspective, the analysis of individual ROH profiles may therefore represent a valuable tool for management strategies. Animals showing high levels of genomic homozygosity could be assigned lower mating priority or excluded from breeding schemes to minimize further losses of genetic diversity (Mastrangelo et al., 2018a). Indeed, long ROH can be enriched in genomic regions that carry deleterious mutations (Szpiech et al., 2013).

OAR1 displayed the highest number of ROH, a pattern attributable primarily to its status as the longest chromosome in the sheep genome, which naturally increases the probability of detecting homozygous segments. The absence of species-wide ROH islands on OAR1 suggests that homozygosity on this chromosome is unevenly distributed across individuals and not the result of strong, shared selective sweeps across breeds. In contrast, the presence of clear ROH islands on OAR2, OAR6 and OAR10, as also reported in other sheep breeds (Al-Mamun et al., 2015a; Manunza et al., 2016; Dzomba et al., 2021; Chessari et al., 2023a), indicates regions that have undergone consistent selection pressures. These genomic regions have been repeatedly identified not only through ROH-based analyses but also using complementary approaches, such as the F_{ST} -outlier method (Kijas et al., 2012; Megdiche et al., 2019), further supporting their role as hotspots of selection in the ovine genome. Additional support for the biological relevance of these genomic hotspots comes from a large comparative study of 100 sheep breeds worldwide (Gorssen et al., 2021), which reported an overlapping selection signature on OAR6 in 15 breeds specialized in wool (e.g., Dorset Horn, Île de France, Merinizzata Italiana, Romanov, Scottish Blackface). Notably, this region harbors QTL related to growth and bone development. Further studies (He et al., 2020; Selli et al., 2021) have also identified a ROH hotspot on this region in Merino-derived breeds, providing additional

support for the relevance of this signal. The ROH island encompassing *FAM184B*, *DCAF16*, *NCAPG*, and *LCORL* corresponds to a genomic region repeatedly identified within ROH islands across livestock species as a target of selection. *FAM184B* has been reported to influence average daily gain and carcass weight in sheep (Yuan et al., 2021; Chessari et al., 2023a; Li et al., 2023). Similarly, *DCAF16* has been highlighted as a relevant gene involved in determining body weight and body conformation in both sheep and goat (Al-Mamun et al., 2015b; Li et al., 2023; Senczuk et al., 2024). In particular, the *NCAPG–LCORL* locus has been consistently associated with variation in body size, growth, and skeletal development, traits that have historically been subject to artificial selection in sheep (Rochus et al., 2018; Yurchenko et al., 2019; Posbergh and Huson, 2020; Ceccobelli et al., 2023; Falchi et al., 2025). In the Gentile di Puglia breed, which has been traditionally selected for production-related and morphological traits typical of Merino-derived populations, the presence of this ROH island likely reflects long-term directional selection acting on growth and body conformation rather than recent demographic events alone.

The most recurrent ROH island in our dataset was located on OAR19, spanning approximately 1.5 Mb (0.38-1.88 Mb) and appearing in nearly 35% of all individuals. This region harbors several genes of interest, including *SLC4A7*, previously associated with greater compensatory gain after fasting and identified as a central component of metabolic pathways related to backfat thickness in cattle (Martins et al., 2021), and *NEK10*, a gene implicated in intramuscular fat content in pigs (Wang et al., 2022) and associated with reproductive traits in Hu sheep (Zhou et al., 2024). Interestingly, the close region (1.91-2.46 Mb) contained only one gene, *EOMES*, which is highly involved in immune response in hot and dry environments in Iranian sheep (Saadatabadi et al., 2023). The ROH island on OAR10 (43-45 Mb) had already been reported in a study of diverse sheep breeds selected for wool productivity and body size (Ma et al., 2025). Within the second ROH island on OAR10 (36-41 Mb), the gene *PCDH9* was identified, previously highlighted as a strong candidate in goats and sheep adapted to hot and arid environments. (Kim et al. (2016). The ROH island on OAR2 (113-117 Mb) contained *CYFIP1*, a gene shown to regulate growth traits in cattle (Purfield et al., 2019), and included carcass traits-related *TUBGCP5* gene (Kenny et al., 2022; Colombi et al., 2024); notably, both genes have also been associated with stress and immune responses (Abied et al., 2020; Wang et al., 2025).

Beyond regions of homozygosity, we also investigated HRR, which provides complementary information into genomic areas potentially involved in fitness and adaptive processes (Chessari et al., 2024; Biscarini et al., 2026). Although HRR have been less extensively characterized in livestock compared with ROH islands, available studies across several species consistently report that, within the same individuals, HRR segments are fewer and shorter than ROH (Ferenčaković et al., 2016; Biscarini et al., 2020; Santos et al., 2021; Selli et al., 2021; Ruan et al., 2022; Chessari et al., 2024). Despite the limited attention given to HRR in livestock, this study provides novel insights into HRR patterns in sheep. We observed a comparable mean number of HRRs across individuals, together with a relatively low standard deviation. Compared with other studies on sheep breeds (Tsartsianidou et al., 2021; Szmatala et al., 2025), our N_{HRR} values were also low, likely due to the detection parameters used. Specifically, these parameters excluded opposite and missing genotype calls, which are among the factors most strongly influencing the

number of detected HRR (Chessari et al., 2024). As a result, only very short HRR segments, all shorter than 1 Mb, were identified. Their limited extent in our dataset suggests the absence of strong, recent selective pressures favoring heterozygous genotypes, mirroring the ROH-based evidence of predominantly ancient inbreeding. This pattern reflects the maintenance of heterozygosity in restricted genomic regions, likely shaped by long-term evolutionary processes rather than by recent directional selection. Indeed, the diversity coefficient (D_{HRR}) estimated at the chromosome level did not simply reflect the inverse of ROH-based inbreeding. Shorter chromosomes, which exhibited higher F_{ROH} values, also showed markedly higher D_{HRR} values (> 0.010), reflecting a heterogeneous genomic architecture characterized by the coexistence of homozygous and heterozygous stretches. In contrast, the first three longest chromosomes displayed consistently low D_{HRR} values (0.003-0.004), suggesting a more homogeneous distribution of genetic variability along their length.

The two HRR islands, each containing at least one annotated gene, are both ~ 0.4 Mb in length, consistent with the expected size range of these hotspot types. The *VPS13B* gene on OAR9 is a well-known candidate involved in adaptation to hot environments (Amiri Ghanatsaman et al., 2023) and has been reported under selection in Mediterranean sheep (Yang et al., 2016) and goats (Serranito et al., 2021). Moreover, *VPS13B* is particularly noteworthy, as it has been previously identified within HRR islands in other local Southern Italian sheep breeds (Carta et al., 2026), suggesting a conserved role in environmental adaptation. Genes identified in OAR2 included *CLCN3* and *NEK1*, which have been implicated in fertility-related functions in humans (Al-Abri et al., 2023; Zhang et al., 2023), while *SH3RF1* has been associated with immune response mechanisms to chronic parasitic infection in Australian sheep (Al Kalaldehy et al., 2019). These findings highlight the functional relevance of HRR islands, suggesting that they may harbor genes contributing to adaptation, reproductive fitness, and disease resistance, all of which are key traits for the conservation and sustainable management of local sheep breeds.

Moreover, it is worth noting the detection of overlap between ROH and HRR, observed to some extent in the OAR10 island. This pattern can arise when natural or artificial selective pressures act differently on distinct subpopulations, causing the same genomic region to become highly homozygous in some individuals while remaining heterozygous in others (Carta et al., 2026; Biscarini et al., 2026). Such opposing trends highlight the complex interplay between selection, population structure, and local adaptation, and underscore the importance of considering both ROH and HRR to fully capture the breed's genomic landscape.

Implications for conservation and breeding strategies

In recent decades, valuable local genetic resources have been lost due to genetic erosion and the widespread use of improved and industrial breeds (Taberlet et al., 2011). From a conservation and management perspective, the results of this study provide several actionable insights for the Gentile di Puglia breed. Although the breed retains an overall satisfactory level of genetic diversity, the clear genetic structuring observed among farms highlights the need for coordinated management at the breed level. In particular, structured mating schemes based on controlled ram exchange among farms could help maintain genetic connectivity and limit the local accumulation of inbreeding, while preserving farm-specific genetic resources. At the

same time, genetically distinct nuclei such as F5 and F7 should be considered priority components of conservation programs, as they represent valuable reservoirs of breed-specific variation. Their management should therefore aim to preserve their genetic identity while avoiding excessive isolation. In parallel, genomic information could be used to optimize breeding decisions at the individual level: animals showing higher genomic homozygosity, especially those carrying long ROH segments, should be used with caution, whereas less autozygous individuals may be preferentially selected to maintain diversity and reduce the risk of further inbreeding. This information should also be integrated into both *ex-situ* and *in-situ* conservation strategies already implemented for some Apulian stocks (Temerario et al., 2023).

Overall, these findings support the integration of genomic data into routine conservation and breeding plans for the Gentile di Puglia breed, with the dual objective of preserving its genetic distinctiveness and ensuring its long-term sustainability.

Conclusions

The extensive sampling in this study enabled a robust and representative assessment of genomic diversity and population structure within the Gentile di Puglia breed, providing a comprehensive overview of its current genetic status. Integrating genomic data with contextual information on breeding practices and known management histories proved essential for interpreting patterns of relatedness and gene flow across farms.

From a conservation perspective, the observed internal structuring represents both a valuable reservoir of genetic diversity and a potential management challenge. While genetically differentiated nuclei contribute to maintaining overall breed variability and effective population size, restricted gene flow may favor the accumulation of runs of homozygosity and increased inbreeding at the farm level. Functional annotation of highly recurrent ROH and HRR islands suggests that selection has targeted genomic regions linked to adaptation, reproduction, immunity, and production traits.

Within the broader context of Merino-type sheep, the Gentile di Puglia breed appears to retain a distinctive genomic identity shaped by both historical selection for wool production and local adaptation. This highlights its value not only as a genetic resource for conservation, but also as a representative of a specific branch within the Merino lineage, preserving genetic features that may have been partially eroded in more intensively selected populations. These findings have direct implications for the current breeding program of the breed. The integration of genomic information into selection schemes can support structured mating strategies, including controlled ram exchange among farms and the selection of breeding animals based on genomic inbreeding and diversity metrics. Such approaches are essential to maintain genetic variability while preserving economically and functionally important traits.

Overall, this study demonstrates how genomic tools can effectively support both conservation and breeding strategies, contributing to the sustainable management of the Gentile di Puglia breed. More broadly, it highlights the value of large-scale genomic analyses as a framework for the long-term conservation and resilience of local livestock breeds in changing environmental and production contexts.

Ethics approval

Blood samples were collected in compliance with the European rules [Council Regulation (EC) No. 1/2005 and Council Regulation (EC) No. 1099/2009] during routine health controls by the public veterinary service. Moreover, the animal study protocol was approved by the Bioethics Committee of the University of Palermo: protocol code UNPA-CLE-98597.

Data and model availability statement

None of the data were deposited in an official repository. Information can be made available from the authors upon request.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

Acknowledgements

The authors gratefully acknowledge all the breeders for their cooperation, availability, and for their contribution to the collection of biological material.

Financial support statement

This research was financed by the project Valorizzazione del germoplasma Ovino mediante tecniche Omiche per il miglioramento della qualità della Lana, VOOL - "AGRITECH - National Research Centre for Agricultural Technologies", CUP H93C22000440007 - PNRR, Missione 4, Componente 2, Investimento 1.4 – finanziato dall'Unione europea – NextGenerationEU. The present study was also supported by the project "Biodiversity and enhancement of local sheep and goat genotypes with a prevalent aptitude for milk production (Acronym: LOCAL)" and the project "Meat Zootechnic biodiversity: recovery and valorization" (Acronym: Bio.Zoo.Ca.Re.), both funded by the Apulian Government through the Rural Development Program PSR PUGLIA 2014-2022 (Measure 10.2).

References

- Abdoli, R., Mirhoseini, S.Z., Ghavi Hossein-Zadeh, N., Zamani, P., Moradi, M.H., Ferdosi, M.H., Sargolzaei, M., Gondro, C., 2023. Runs of homozygosity and cross-generational inbreeding of Iranian fat-tailed sheep. *Heredity* 130, 358-367.
- Abied, A., Xu, L., Sahlu, B.W., Xing, F., Ahbara, A., Pu, Y., Lin, J., Berihulay, H., Islam, R., He, X., Mwacharo, J.M., Zhao, Q., Ma, Y., 2020. Genome-wide analysis revealed homozygosity and demographic history of five chinese sheep breeds adapted to different environments. *Genes* 11, 1480-1496.
- Al-Abri, M., Kharousi, K.A., Hamrashdi, A.A., Toobi, A.G.A., Salem, M.M.I., 2023. Genome wide association analysis for twinning ability in Jabal Akhdar Omani goats. *Small Ruminant Research* 221, 106951.
- Al-Mamun, H.A., Clark, S.A., Kwan, P., Gondro, C., 2015a. Genome-wide linkage disequilibrium and genetic diversity in five populations of Australian domestic sheep. *Genetics Selection Evolution* 47, 90-104.
- Al-Mamun, H.A., Kwan, P., Clark, S.A., Ferdosi, M.H., Tellam, R., Gondro, C., 2015b. Genome-wide association study of body weight in Australian Merino sheep reveals an orthologous region on OAR6 to human and bovine genomic regions affecting height and weight. *Genetics Selection Evolution* 47, 66-77.
- Al Kalaldehy, M., Gibson, J., Lee, S.H., Gondro, C., van der Werf, J.H.J., 2019. Detection of genomic regions underlying resistance to gastrointestinal parasites in Australian sheep. *Genetics Selection Evolution* 51, 37-55.
- Alexander, D.H., Novembre, J., Lange, K., 2009. Fast model-based estimation of ancestry in unrelated individuals. *Genome Research* 19, 1655-1664.
- Amiri Ghanatsaman, Z., Ayatollahi Mehrgardi, A., Asadollahpour Nanaei, H., Esmailizadeh, A., 2023. Comparative genomic analysis uncovers candidate

- genes related with milk production and adaptive traits in goat breeds. *Scientific Reports* 13, 8722-8732.
- Baazaoui, I., Bedhiaf-Romdhani, S., Mastrangelo, S., Ciani, E., 2021. Genome-wide analyses reveal population structure and identify candidate genes associated with tail fatness in local sheep from a semi-arid area. *Animal* 15, 100193.
- Bakoev, S., Kolosova, M., Romanets, T., Bakoev, F., Kolosov, A., Romanets, E., Korobeinikova, A., Bakoeva, I., Akhmedli, V., Getmantseva, L., 2025. Visualization of runs of homozygosity and classification using convolutional neural networks. *Biology (Basel)* 14, 426-437.
- Barbato, M., Orozco-terWengel, P., Tapio, M., Bruford, M.W., 2015. SNeP: a tool to estimate trends in recent effective population size trajectories using genome-wide SNP data. *Frontiers in Genetics* 6, 109.
- Biscarini, F., Cozzi, P., Gaspa, G., Marras, G., 2018. detectRUNS: an R package to detect runs of homozygosity and heterozygosity in diploid genomes. Retrieved on 20 October 2025 from <https://orca.cardiff.ac.uk/108906/>.
- Biscarini, F., Mastrangelo, S., Catillo, G., Senczuk, G., Ciampolini, R., 2020. Insights into genetic diversity, runs of homozygosity and heterozygosity-rich regions in Maremmana semi-feral cattle using pedigree and genomic data. *Animals (Basel)* 10, 2285-2302.
- Biscarini, F., Cesarani, A., Criscione, A., Mastrangelo, S., 2026. Invited review: the application of runs of homozygosity and heterozygosity-rich regions to infer population history in livestock species. *Livestock Science* 307, 105950.
- Bordonaro, S., Chessari, G., Mastrangelo, S., Senczuk, G., Chessa, S., Castiglioni, B., Tumino, S., Marletta, D., Criscione, A., 2023. Genome-wide population structure, homozygosity, and heterozygosity patterns of Nero Siciliano pig in the framework of Italian and cosmopolitan breeds. *Animal Genetics* 54, 591–605.
- Carta, F., Chessari, G., Sardina, M.T., Riggio, S., Senczuk, G., Cesarani, A., Criscione, A., Mastrangelo, S., 2026. Detection of homozygosity and heterozygosity regions in mediterranean sheep breeds revealed by high-density SNP array. *Journal of Animal Science* 104, skag014.
- Ceccobelli, S., Landi, V., Senczuk, G., Mastrangelo, S., Sardina, M.T., Ben-Jemaa, S., Persichilli, C., Karsli, T., Balteanu, V.A., Raschia, M.A., Poli, M.A., Ciappesoni, G., Muchadeyi, F.C., Dzomba, E.F., Kunene, N.W., Lühken, G., Deniskova, E.V., Dotsev, A.V., Zinovieva, N.A., Zsolnai, A., Anton, I., Kusza, S., Carolino, N., Santos-Silva, F., Kawęcka, A., Świątek, M., Niznikowski, R., Spehar, M., Anaya, G., Granero, A., Perloiro, T., Cardoso, P., Grande, S., López de los Santos, B., Danchin-Burge, C., Pasquini, M., Martínez Martínez, A., Bermejo, J.V.D., Lasagna, E., Ciani, E., Sarti, F.M., Pilla, F., 2023. A comprehensive analysis of the genetic diversity and environmental adaptability in worldwide Merino and Merino-derived sheep breeds. *Genetics Selection Evolution* 55, 24-42.

- Chang, C.C., Chow, C.C., Tellier, L.C., Vattikuti, S., Purcell, S.M., Lee, J.J., 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience* 4, 1-16.
- Chessari, G., Criscione, A., Marletta, D., Crepaldi, P., Portolano, B., Manunza, A., Cesarani, A., Biscarini, F., Mastrangelo, S., 2024. Characterization of heterozygosity-rich regions in Italian and worldwide goat breeds. *Scientific Reports* 14, 1-16.
- Chessari, G., Criscione, A., Tolone, M., Bordonaro, S., Rizzuto, I., Riggio, S., Macaluso, V., Moscarelli, A., Portolano, B., Sardina, M.T., Mastrangelo, S., 2023a. High-density SNP markers elucidate the genetic divergence and population structure of Noticiana sheep breed in the Mediterranean context. *Frontiers in Veterinary Science* 10, 1127354.
- Chessari, G., Criscione, A., Tolone, M., Zumbo, A., Riggio, S., Rizzuto, I., Moscarelli, A., Macaluso, V., Portolano, B., Sardina, M.T., Mastrangelo, S., 2023b. Genome wide analyses reveal the population distinctiveness of the Nera del Mela sheep. *Italian Journal of Animal Science* 22, 548-559.
- Ciani, E., Ciampolini, R., D'Andrea, M., Castellana, E., Cecchi, F., Incoronato, C., d'Angelo, F., Albenzio, M., Pilla, F., Matassino, D., Cianci, D., 2013. Analysis of genetic variability within and among Italian sheep breeds reveals population stratification and suggests the presence of a phylogeographic gradient. *Small Ruminant Research* 112, 21-27.
- Ciani, E., Crepaldi, P., Nicoloso, L., Lasagna, E., Sarti, F.M., Moioli, B., Napolitano, F., Carta, A., Usai, G., D'Andrea, M., Marletta, D., Ciampolini, R., Riggio, V., Occidente, M., Matassino, D., Kompan, D., Modesto, P., Macciotta, N., Ajmone-Marsan, P., Pilla, F., 2014. Genome-wide analysis of Italian sheep diversity reveals a strong geographic pattern and cryptic relationships between breeds. *Animal Genetics* 45, 256-266.
- Ciani, E., Lasagna, E., D'Andrea, M., Alloggio, I., Marroni, F., Ceccobelli, S., Delgado Bermejo, J.V., Sarti, F.M., Kijas, J., Lenstra, J.A., Pilla, F., International Sheep Genomics, C., 2015. Merino and Merino-derived sheep breeds: a genome-wide intercontinental study. *Genetics Selection Evolution* 47, 1-11.
- Colombi, D., Rovelli, G., Luigi-Sierra, M.G., Ceccobelli, S., Guan, D., Perini, F., Sbarra, F., Quaglia, A., Sarti, F.M., Pasquini, M., Amills, M., Lasagna, E., 2024. Population structure and identification of genomic regions associated with productive traits in five Italian beef cattle breeds. *Scientific Reports* 14, 8529-8539.
- d'Angelo, F., Albenzio, M., Sevi, A., Ciampolini, R., Cecchi, F., Ciani, E., Muscio, A., 2009. Genetic variability of the Gentile di Puglia sheep breed based on microsatellite polymorphism. *Journal of Animal Science* 87, 1205-1209.
- Deniskova, T., Dotsev, A., Selionova, M., Brem, G., Zinovieva, N., 2021. Biodiversity of Russian local sheep breeds based on pattern of runs of homozygosity. *Diversity* 13, 360-375.

- Dzomba, E.F., Chimonyo, M., Pierneef, R., Muchadeyi, F.C., 2021. Runs of homozygosity analysis of South African sheep breeds from various production systems investigated using OvineSNP50k data. *BMC Genomics* 22, 1-14.
- Eusebi, P.G., Martinez, A., Cortes, O., 2019. Genomic tools for effective conservation of livestock breed diversity. *Diversity* 12, 1-16.
- Excoffier, L., Lischer, H.E., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10, 564-567.
- Falchi, L., Cesarani, A., Criscione, A., Hidalgo, J., Garcia, A., Mastrangelo, S., Macciotta, N.P.P., 2024. Effect of genotyping density on the detection of runs of homozygosity and heterozygosity in cattle. *Journal of Animal Science* 102, skae147.
- Falchi, L., Rochus, C.M., Špehar, M., Ramljak, J., Kasap, A., Macciotta, N.P.P., Pocrnic, I., Cesarani, A., 2025. Genetic diversity and selection signatures in related Mediterranean breeds: Sarda, Pag, and Istrian sheep. *Small Ruminant Research* 253, 107654.
- Ferenčaković, M., Banadinović, M., Mercvajler, M., Khayat Zadeh, N., Mészáros, G., Cubric-Curik, V., Curik, I., Sölkner, J., 2016. Mapping of heterozygosity rich regions in Austrian pinzgauer cattle. *Acta Agriculturae Slovenica* 5, S41-44.
- Ferenčaković, M., Sölkner, J., Curik, I., 2013. Estimating autozygosity from high-throughput information: effects of SNP density and genotyping errors. *Genetics Selection Evolution* 45, 1-9.
- Giovannini, S., Chessari, G., Riggio, S., Marletta, D., Sardina, M.T., Mastrangelo, S., Sarti, F.M., 2024. Insight into the current genomic diversity, conservation status and population structure of Tunisian Barbarine sheep breed. *Frontiers in Genetics* 15, 1379086.
- Gorsen, W., Meyermans, R., Janssens, S., Buys, N., 2021. A publicly available repository of ROH islands reveals signatures of selection in different livestock and pet species. *Genetics Selection Evolution* 53, 1-10.
- Gurgul, A., Jasielczuk, I., Miksza-Cybulska, A., Kawęcka, A., Szmatoła, T., Krupiński, J., 2021. Evaluation of genetic differentiation and genome-wide selection signatures in Polish local sheep breeds. *Livestock Science* 251, 104635.
- Hampton, J.O., MacKenzie, D.I., Forsyth, D.M., 2019. How many to sample? Statistical guidelines for monitoring animal welfare outcomes. *PLoS ONE* 14, e0211417.
- Han, Z.P., Yang, R.Z., Zhou, W., Zhang, L.L., Wang, J.R., Liu, C.J., Liu, S.D., 2024. Population structure and selection signal analysis of indigenous sheep from the southern edge of the Taklamakan Desert. *BMC Genomics* 25, 681-697.

- He, S., Di, J., Han, B., Chen, L., Liu, M., Li, W., 2020. Genome-wide scan for runs of homozygosity identifies candidate genes related to economically important traits in Chinese Merino. *Animals (Basel)* 10, 524-537.
- Hoban, S., Paz-Vinas, I., Aitken, S., Bertola, L.D., Breed, M.F., Bruford, M.W., Funk, W.C., Grueber, C.E., Heuertz, M., Hohenlohe, P., Hunter, M.E., Jaffé, R., Fernandes, M.L., Mergeay, J., Moharrek, F., O'Brien, D., Segelbacher, G., Vernesi, C., Waits, L., Laikre, L., 2021. Effective population size remains a suitable, pragmatic indicator of genetic diversity for all species, including forest trees. *Biological Conservation* 253, 108906.
- Kenny, D., Carthy, T.R., Murphy, C.P., Sleator, R.D., Evans, R.D., Berry, D.P., 2022. The association between genomic heterozygosity and carcass merit in cattle. *Frontiers in Genetics* 13, 789270.
- Kijas, J.W., Lenstra, J.A., Hayes, B., Boitard, S., Porto Neto, L.R., San Cristobal, M., Servin, B., McCulloch, R., Whan, V., Gietzen, K., Paiva, S., Barendse, W., Ciani, E., Raadsma, H., McEwan, J., Dalrymple B., International Sheep Genomics Consortium. 2012. Genome-wide analysis of the world's sheep breeds reveals high levels of historic mixture and strong recent selection. *PLoS Biology* 10, e1001258.
- Kim, E.S., Elbeltagy, A.R., Aboul-Naga, A.M., Rischkowsky, B., Sayre, B., Mwacharo, J.M., Rothschild, M.F., 2016. Multiple genomic signatures of selection in goats and sheep indigenous to a hot arid environment. *Heredity (Edinb)* 116, 255-264.
- Kriaridou, C., Tsairidou, S., Frasin, C., Gorjanc, G., Looseley, M.E., Johnston, I.A., Houston, R.D., Robledo, D., 2023. Evaluation of low-density SNP panels and imputation for cost-effective genomic selection in four aquaculture species. *Frontiers in Genetics* 14, 1194266.
- Li, C., Li, J., Wang, H., Zhang, R., An, X., Yuan, C., Guo, T., Yue, Y., 2023. Genomic selection for live weight in the 14th month in Alpine Merino sheep combining GWAS information. *Animals (Basel)* 13, 3516-3531.
- Ma, R., Liu, J., Ma, X., Yang, J., 2025. Genome-wide runs of homozygosity reveal inbreeding levels and trait-associated candidate genes in diverse sheep breeds. *Genes (Basel)* 16, 316-336.
- Manunza, A., Cardoso, T.F., Noce, A., Martinez, A., Pons, A., Bermejo, L.A., Landi, V., Sanchez, A., Jordana, J., Delgado, J.V., Adan, S., Capote, J., Vidal, O., Ugarte, E., Arranz, J.J., Calvo, J.H., Casellas, J., Amills, M., 2016. Population structure of eleven Spanish ovine breeds and detection of selective sweeps with BayeScan and hapFLK. *Scientific Reports* 6, 1-10.
- Martins, R., Machado, P.C., Pinto, L.F.B., Silva, M.R., Schenkel, F.S., Brito, L.F., Pedrosa, V.B., 2021. Genome-wide association study and pathway analysis for fat deposition traits in nellore cattle raised in pasture-based systems. *Journal of Animal Breeding and Genetics* 138, 360-378.

- Mastrangelo, S., Ciani, E., Sardina, M.T., Sottile, G., Pilla, F., Portolano, B., Bi.Ov. Ita, C., 2018a. Runs of homozygosity reveal genome-wide autozygosity in Italian sheep breeds. *Animal Genetics* 49, 71-81.
- Mastrangelo, S., Di Gerlando, R., Tolone, M., Tortorici, L., Sardina, M.T., Portolano, B., International Sheep Genomics, C., 2014. Genome wide linkage disequilibrium and genetic structure in Sicilian dairy sheep breeds. *BMC Genetics* 15, 1-10.
- Mastrangelo, S., Sardina, M.T., Tolone, M., Di Gerlando, R., Sutera, A.M., Fontanesi, L., Portolano, B., 2018b. Genome-wide identification of runs of homozygosity islands and associated genes in local dairy cattle breeds. *Animal* 12, 2480-2488.
- McKay, S.D., Schnabel, R.D., Murdoch, B.M., Matukumalli, L.K., Aerts, J., Coppieters, W., Crews, D., Dias Neto, E., Gill, C.A., Gao, C., Mannen, H., Stothard, P., Wang, Z., Van Tassell, C.P., Williams, J.L., Taylor, J.F., Moore, S.S., 2007. Whole genome linkage disequilibrium maps in cattle. *BMC Genetics* 8, 74-86.
- McLaren, W., Gil, L., Hunt, S.E., Riat, H.S., Ritchie, G.R., Thormann, A., Flicek, P., Cunningham, F., 2016. The Ensembl Variant Effect Predictor. *Genome Biology* 17, 122-136.
- McQuillan, R., Leutenegger, A.L., Abdel-Rahman, R., Franklin, C.S., Pericic, M., Barac-Lauc, L., Smolej-Narancic, N., Janicijevic, B., Polasek, O., Tenesa, A., Macleod, A.K., Farrington, S.M., Rudan, P., Hayward, C., Vitart, V., Rudan, I., Wild, S.H., Dunlop, M.G., Wright, A.F., Campbell, H., Wilson, J.F., 2008. Runs of homozygosity in European populations. *American Journal of Human Genetics* 83, 359-372.
- Megdiche, S., Mastrangelo, S., Ben Hamouda, M., Lenstra, J.A., Ciani, E., 2019. A combined multi-cohort approach reveals novel and known genome-wide selection signatures for wool traits in Merino and Merino-Derived sheep breeds. *Frontiers in Genetics* 10, 1025.
- Milanesi, M., Capomaccio, S., Vajana, E., Bomba, L., Garcia, J.F., Ajmone-Marsan, P., Colli, L., 2017. BITE: an R package for biodiversity analyses. Retrieved on 20 October 2025 from <https://doi.org/10.1101/181610>.
- Moioli, B., Napolitano, F., Orrù, L., Catillo, G., 2006. Analysis of the genetic diversity between Gentile di Puglia, Sopravissana and Sarda sheep breeds using microsatellite markers. *Italian Journal of Animal Science* 5, 73-78.
- Nani, J.P., Penagaricano, F., 2020. Whole-genome homozygosity mapping reveals candidate regions affecting bull fertility in US Holstein cattle. *BMC Genomics* 21, 338-347.
- Neuditschko, M., Khatkar, M.S., Raadsma, H.W., 2012. NetView: a high-definition network-visualization approach to detect fine-scale population structures from genome-wide patterns of variation. *PLoS ONE* 7, e48375.

- Peripolli, E., Munari, D.P., Silva, M., Lima, A.L.F., Irgang, R., Baldi, F., 2017. Runs of homozygosity: current knowledge and applications in livestock. *Animal Genetics* 48, 255–271.
- Posbergh, C.J., Huson, H.J., 2020. All sheeps and sizes: a genetic investigation of mature body size across sheep breeds reveals a polygenic nature. *Animal Genetics* 52, 99-107.
- Purfield, D.C., Evans, R.D., Berry, D.P., 2019. Reaffirmation of known major genes and the identification of novel candidate genes associated with carcass-related metrics based on whole genome sequence within a large multi-breed cattle population. *BMC Genomics* 20, 720-737.
- Purfield, D.C., McParland, S., Wall, E., Berry, D.P., 2017. The distribution of runs of homozygosity and selection signatures in six commercial meat sheep breeds. *PLoS ONE* 12, e0176780.
- Rafter, P., McHugh, N., Pabiou, T., Berry, D.P., 2022. Inbreeding trends and genetic diversity in purebred sheep populations. *Animal* 16, 100604.
- Ramljak, J., Spehar, M., Ceranac, D., Drzaic, V., Pocrnic, I., Barac, D., Mioc, B., Siric, I., Barac, Z., Ivankovic, A., Kasap, A., 2024. Genomic characterization of local Croatian sheep breeds-effective population size, inbreeding & signatures of selection. *Animals (Basel)* 14, 1928-1944.
- RCoreTeam, 2025. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rochus, C.M., Tortereau, F., Plisson-Petit, F., Restoux, G., Moreno-Romieux, C., Tosser-Klopp, G., Servin, B., 2018. Revealing the selection history of adaptive loci using genome-wide scans for selection: an example from domestic sheep. *BMC Genomics* 19, 71-88.
- Ruan, D., Yang, J., Zhuang, Z., Ding, R., Huang, J., Quan, J., Gu, T., Hong, L., Zheng, E., Li, Z., Cai, G., Wang, X., Wu, Z., 2022. Assessment of heterozygosity and genome-wide analysis of heterozygosity regions in two Duroc pig populations. *Frontiers in Genetics* 12, 1-12.
- Saadatabadi, L.M., Mohammadabadi, M., Nanaei, H.A., Ghanatsaman, Z.A., Stavetska, R.V., Kalashnyk, O., Kochuk-Yashchenko, O.A., Kucher, D.M., 2023. Unraveling candidate genes related to heat tolerance and immune response traits in some native sheep using whole genome sequencing data. *Small Ruminant Research* 225, 107018.
- Salehi, R., Javanmard, A., Mokhber, M., Alijani, S., 2025. Estimating linkage disequilibrium and effective population size across generations in Holstein cattle. *Veterinary Medicine and Science* 11, e70684.
- Santillo, A., Della Malva, A., Albenzio, M., 2025. Preserving biodiversity of sheep and goat farming in the Apulia region. *Animals (Basel)* 15, 1610-1630.

- Santos, W.B., Pimenta Schettini, G., Fonseca, M.G., Pereira, G.L., Loyola Chardulo, L.A., Rodrigues Machado Neto, O., Baldassini, W.A., Nunes de Oliveira, H., Abdallah Curi, R., 2021. Fine-scale estimation of inbreeding rates, runs of homozygosity and genome-wide heterozygosity levels in the Mangalarga Marchador horse breed. *Journal of Animal Breeding and Genetics* 138, 161-173.
- Sarti, F.M., Lasagna, E., Panella, F., Lebboroni, G., Renieri, C., 2006. Wool quality in Gentile di Puglia sheep breed as measure of genetic integrity. *Italian Journal of Animal Science* 5, 371-376.
- Selli, A., Ventura, R.V., Fonseca, P.A.S., Buzanskas, M.E., Andrietta, L.T., Balieiro, J.C.C., Brito, L.F., 2021. Detection and visualization of heterozygosity-rich regions and runs of homozygosity in worldwide sheep populations. *Animals (Basel)* 11, 2696-2720.
- Senczuk, G., Macri, M., Di Civita, M., Mastrangelo, S., Del Rosario Fresno, M., Capote, J., Pilla, F., Delgado, J.V., Amills, M., Martinez, A., 2024. The demographic history and adaptation of Canarian goat breeds to environmental conditions through the use of genome-wide SNP data. *Genetics Selection Evolution* 56, 1-15.
- Serranito, B., Cavalazzi, M., Vidal, P., Taurisson-Mouret, D., Ciani, E., Bal, M., Rouvellac, E., Servin, B., Moreno-Romieux, C., Tosser-Klopp, G., Hall, S.J.G., Lenstra, J.A., Pompanon, F., Benjelloun, B., Da Silva, A., 2021. Local adaptations of Mediterranean sheep and goats through an integrative approach. *Scientific Reports* 11, 1-17.
- Signer-Hasler, H., Burren, A., Ammann, P., Drogemuller, C., Flury, C., 2019. Runs of homozygosity and signatures of selection: a comparison among eight local Swiss sheep breeds. *Animal Genetics* 50, 512-525.
- Steinig, E.J., Neuditschko, M., Khatkar, M.S., Raadsma, H.W., Zenger, K.R., 2016. netview p: a network visualization tool to unravel complex population structure using genome-wide SNPs. *Molecular Ecology Resources* 16, 216-227.
- Szmatola, T., Ropka-Molik, K., Jasielczuk, I., Kawecka, A., Gurgul, A., 2025. Exploring runs of homozygosity and heterozygosity in sheep breeds maintained in Poland. *Genes (Basel)* 16, 1-18.
- Taberlet, P., Coissac, E., Pansu, J., Pompanon, F., 2011. Conservation genetics of cattle, sheep, and goats. *Comptes Rendus. Biologies* 334, 247-254.
- Temerario, L., Monaco, D., Mastroiocco, A., Martino, N. A., Cseh, S., Lacalandra, G. M., Ciani, E., Dell'Aquila, M. E., 2023. New strategies for conservation of Gentile di Puglia sheep breed, an autochthonous capital of millennial tradition in Southern Italy. *Animals* 13, 2371.
- Tsartsianidou, V., Sanchez-Molano, E., Kapsona, V.V., Basdagianni, Z., Chatziplis, D., Arsenos, G., Triantafyllidis, A., Banos, G., 2021. A comprehensive genome-wide scan detects genomic regions related to local adaptation and climate

- resilience in Mediterranean domestic sheep. *Genetics Selection Evolution* 53, 1-17.
- Wang, L., Xie, Y., Chen, W., Zhang, Y., Zeng, Y., 2022. Identification and functional prediction of long noncoding RNAs related to intramuscular fat content in Laiwu pigs. *Animal Bioscience* 35, 115-125.
- Wang, S., Liang, L., Ziyayiding, D., Jiao, W., Kasimu, H., He, S., Liu, M., 2025. Inbreeding patterns and genetic diversity under selection in Teha sheep. *Frontiers in Genetics* 16, 1576125.
- Weigel, K.A., 2001. Controlling inbreeding in modern breeding programs. *Journal of Dairy Science* 84, 177-184.
- Wickham, H., 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York, NY, USA. url:<https://ggplot2.tidyverse.org>.
- Yang, J., Li, W.R., Lv, F.H., He, S.G., Tian, S.L., Peng, W.F., Sun, Y.W., Zhao, Y.X., Tu, X.L., Zhang, M., Xie, X.L., Wang, Y.T., Li, J.Q., Liu, Y.G., Shen, Z.Q., Wang, F., Liu, G.J., Lu, F., Kantanen, J., Han, J.-J., Li, M.H., Liu, M.J., 2016. Whole-Genome sequencing of native sheep provides insights into rapid adaptations to extreme environments. *Molecular Biology and Evolution* 33, 2576-2592.
- Yuan, Z., Sunduimijid, B., Xiang, R., Behrendt, R., Knight, M.I., Mason, B.A., Reich, C.M., Prowse-Wilkins, C., Vander Jagt, C.J., Chamberlain, A.J., MacLeod, I.M., Li, F., Yue, X., Daetwyler, H.D., 2021. Expression quantitative trait loci in sheep liver and muscle contribute to variations in meat traits. *Genetics Selection Evolution* 53, 8-22.
- Yurchenko, A.A., Deniskova, T.E., Yudin, N.S., Dotsev, A.V., Khamiruev, T.N., Selionova, M.I., Egorov, S.V., Reyer, H., Wimmers, K., Brem, G., Zinovieva, N.A., Larkin, D.M., 2019. High-density genotyping reveals signatures of selection related to acclimation and economically important traits in 15 local sheep breeds from Russia. *BMC Genomics* 20, 294-213.
- Zhang, Y., Lai, J., Wang, X., Li, M., Zhang, Y., Ji, C., Chen, Q., Lu, S., 2023. Genome-wide single nucleotide polymorphism (SNP) data reveal potential candidate genes for litter traits in a Yorkshire pig population. *Archives Animal Breeding* 66, 357-368.
- Zhou, W., Zhang, C.L., Han, Z., Li, X., Bai, X., Wang, J., Yang, R., Liu, S., 2024. Genome-wide selection reveals candidate genes associated with multiple teats in Hu sheep. *Animal Biotechnology* 35, 2380766.

Tables

Table 1

Genetic diversity indices estimated for the Gentile di Puglia sheep breed (whole dataset). Observed (H_O) and expected (H_E) heterozygosity, overall inbreeding coefficient (F_{IT}), minor allele frequency (MAF) and relative standard deviations (SD) are reported. Moreover, effective population size at 13th generation (Ne^{13}) is provided.

$H_O \pm SD$	$H_E \pm SD$	$F_{IT} \pm SD$	$MAF \pm SD$	Ne^{13}
0.374 ± 0.118	0.383 ± 0.120	0.022 ± 0.036	0.296 ± 0.130	623

Table 2

Runs of homozygosity (ROH) and heterozygosity-rich regions (HRR) summary statistics for the Gentile di Puglia sheep breed. All indices are reported as mean values and standard deviations (SD) over the entire population: the mean number of ROH and HRR per individual (N_{ROH}/N_{HRR}), the mean length of ROH and HRR in Mb per individual (L_{ROH}/L_{HRR}), the total length covered by ROH and HRR segments in Mb (S_{ROH}/S_{HRR}), the population genomic inbreeding coefficient (F_{ROH}) and the genomic diversity coefficient (D_{HRR}). Length classes are also presented as percentage (%) contributions across chromosomes.

Type	Total	$N_{ROH} \pm SD$	$L_{ROH} \pm SD$	$S_{ROH} \pm SD$	$F_{ROH} \pm SD$	Length classes (%)
ROH	31	$23.48 \pm$	$3.51 \pm$	$92.33 \pm$	$0.037 \pm$	1Mb-2Mb: 38.03%
	342	13.58	1.19	72.55	0.029	2Mb-4Mb: 30.81%
						4Mb-8Mb: 20.07%
						8Mb-16Mb: 9.18%
						>16Mb: 1.92%
Type	Total	$N_{HRR} \pm SD$	$L_{HRR} \pm SD$	$S_{HRR} \pm SD$	$D_{HRR} \pm SD$	Length classes (%)

HRR	12 315	9.22 ± 3.07	0.52 ± 0.05	4.79 ± 1.66	0.002 ± 0.001	0.25Mb-0.50Mb: 53.69%
						0.50Mb-1Mb: 45.21%
						1Mb-2Mb: 1.10%

Table 3

Runs of homozygosity (ROH) and heterozygosity-rich regions (HRR) islands identified in the Gentile di Puglia sheep breed. The following information is reported: chromosome (OAR), genomic region positions (in Mega base pairs, Mb), number of Single Nucleotide Polymorphisms (SNP) identified (nSNP), and annotated genes and quantitative trait loci (QTLs) for each island.

Analysis	OAR	Start Mb	End Mb	nSNP	Genes	QTLs
ROH	2	113.99	117.04	46	<i>CYFIP1, TUBGCP5, CCDC115, IMP4, AMER3, PTPN18, ARHGEF4, FAM168B, PLEKHB2</i>	Horn type
	2	123.22	124.51	18	<i>FSIP2</i>	
	6	37.97	39.57	39	<i>FAM184B, DCAF16, NCAPG, LCORL</i>	BW Bone area Total bone Milk fat yield
	9	9.95	10.82	16		Maedi-Visna virus susceptibility

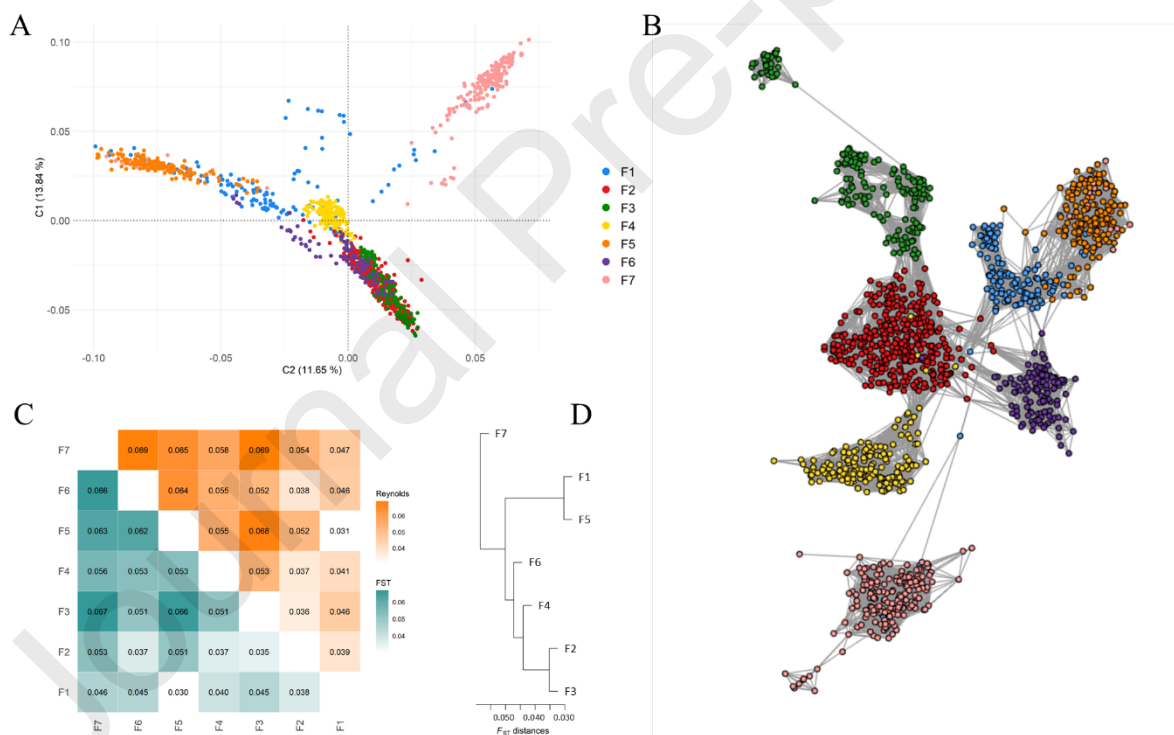
	10	36.57	41.54	54	<i>PSPC1, MPHOSPH8, PARP4, NSD3, CENPJ, RNF17, ATP12A, PCDH9</i>	
	10	43.59	45.44	29	<i>KLHL1</i>	
	19	0.81	1.88	25	<i>UBE2D4, DBNL, VOPP1, LANCL2, EGFR, SEC61G, NEK10, SLC4A7</i>	
	19	1.91	2.46	12	<i>EOMES</i>	
HRR	2	111.49	111.89	10	<i>CLCN3, NEK1, SH3RF1</i>	Horn type Milk fat yield
	3	109.62	110.04	8		
	9	77.67	78.08	8	<i>VPS13B</i>	Milk fat yield
	10	37.08	37.37	7		
	10	42.16	42.88	14		

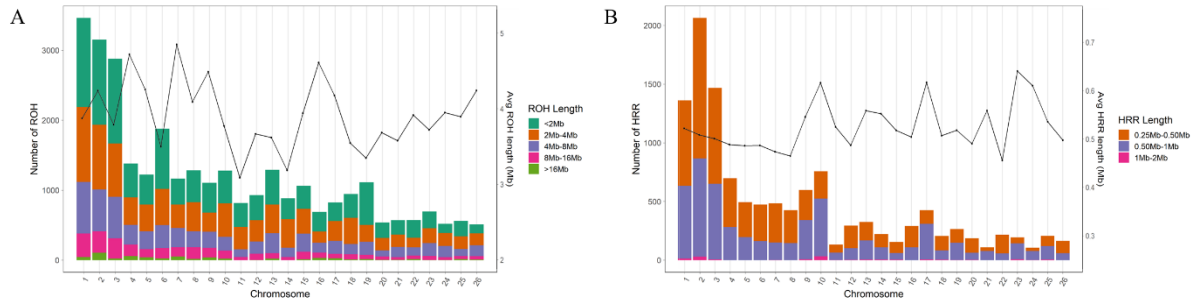
Figure captions

Fig. 1. (A) Multidimensional scaling (MDS) plot of the Gentile di Puglia sheep breed grouped into seven farms, based on components (C)1 and C2; (B) Network visualization of the connectedness among individuals using k-nearest neighbours (K-NN=36); (C) Heatmap of the pairwise genetic distances between farms, where the lower diagonal represents population differentiation index (F_{ST}) distances, while the upper diagonal represents Reynolds distances; (D) Dendrogram based on F_{ST} distances using the average (UPGMA) linkage method.

Fig. 2. Barplot of runs of homozygosity (ROH) (A) and heterozygosity-rich regions (HRR) (B) in the Gentile di Puglia sheep breed. The number of segments and their chromosomal distribution are shown on the left-y axis, while the average length of runs in Mb is reported on the right-y axis. Different bar colors represent length classes according to ROH and HRR classifications.

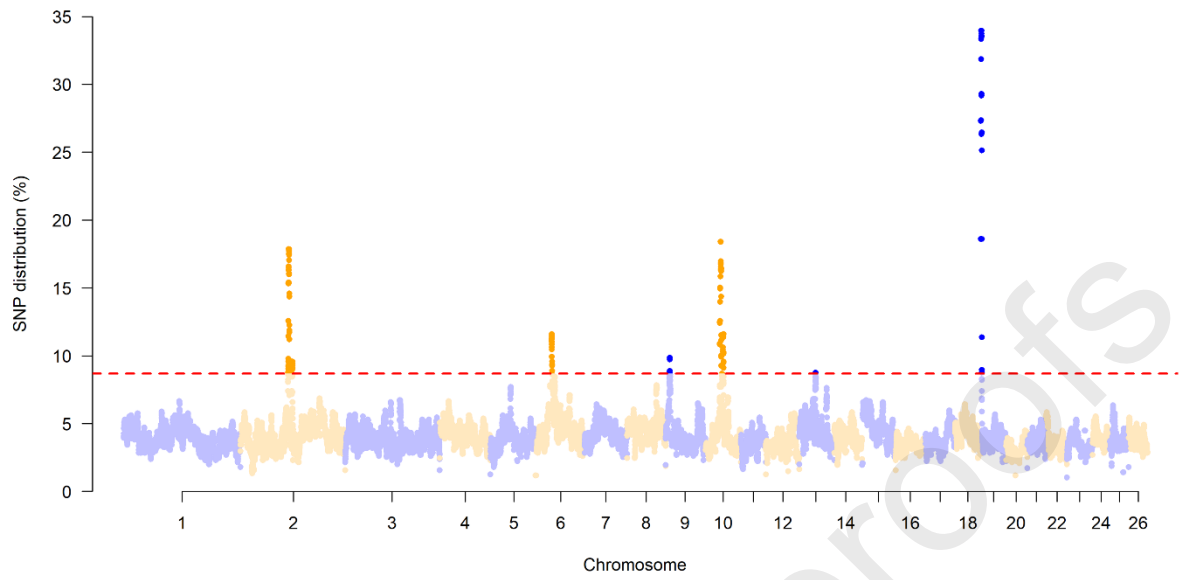
Fig. 3. Manhattan plot showing Single Nucleotide Polymorphisms (SNP) distribution (%) across chromosomes in the Gentile di Puglia sheep breed. Low-percentage points are shaded for clarity, and dashed lines indicate the significance thresholds: (A) 8.68% (top 0.5% of the empirical distribution) for runs of homozygosity (ROH) and (B) 11.89% (top 0.1% of the empirical distribution) for heterozygosity-rich regions (HRR) results.





Journal Pre-proofs

A



B

