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RESEARCH ARTICLE

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Phenotypic and genomic characterization of the Maltese hunting dog (*Kelb tal-Kaċċa ta' Malta*)

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ABSTRACT

The traditional Maltese Hunting Dog (*Kelb tal-Kaċċa ta' Malta*, KTKM) is deeply rooted in Maltese historical and cultural identity, being valued for its versatility in traditional hunting, where it usually flushes out and retrieves small migratory bird species. This study aims to characterise the KTKM phenotypically and genomically, to support its official recognition as a breed and the drafting of a breed standard. Morphological and visual examination of 36 adult KTKM, underline their similarity to European Braque-type dogs, presenting medium-sized and meso-dolicomorphic body, mesocephalic head, large drop ears, thick hanging lips, and a short haired orange-and-white coat. Genomic data of 33 KTKM were compared with 41 dog breeds, including Italian and European breeds belonging to hunting dogs, primitive dogs, terriers, and shepherds. Genomic structure analyses placed the KTKM within the pointing dog cluster, phenotypically and functionally consistent, but also highlighted possible historical links to the *Kelb tal-Fenek* (indigenous to Malta and known internationally as the Pharaoh Hound) and spaniels. Admixture analysis showed high genetic uniformity and uniqueness within the KTKM sampled population, while runs of homozygosity indicated a relatively low inbreeding coefficient. In conclusion, the KTKM can be considered as being a distinct, genetically uniform breed that is aligned with pointing dogs, showing moderate levels of inbreeding and good heterozygosity. Breed recognition and conservation strategies are crucial to safeguard its gene pool and cultural significance.

HIGHLIGHTS

- KTKM are mesocephalic and meso-dolicomorphic.
- Phenotype and genome resemble those of pointing dogs.
- Genetically uniform breed with links to primitive and spaniel dogs.

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Introduction

Hunting has for centuries played a fundamental role in Malta's cultural and rural traditions, with hunting dogs being an essential component that complimented this practice. Historical archival records indicate that hunting with dogs was already well established on the Maltese Islands in the sixteenth century, with legislative measures regulating the activity under the rule of the Order of St. John (Cassar 2018). At that time, hunting primarily involved trapping and archery. To accommodate the knights' recreational pursuits, animals were often imported from abroad and released into the wild, transforming hunting into a sport enjoyed by the nobility. In contrast, for the common people, hunting remained a practical activity, a vital source of free protein. Notably, a decree by Grand Master Ximenes in 1773 referenced the use of the term '*braque*-' type dog for rabbit hunting (https://www.huntinginmalta.org.mt//maltese-hunting-dog). The use of this term, rather than *Cernechi,* that is historically associated with the *Kelb tal-Fenek* also known internationally as the Pharaoh Hound (PHAR), suggests the coexistence of at least two distinct hunting breeds in Malta at the time, likely more

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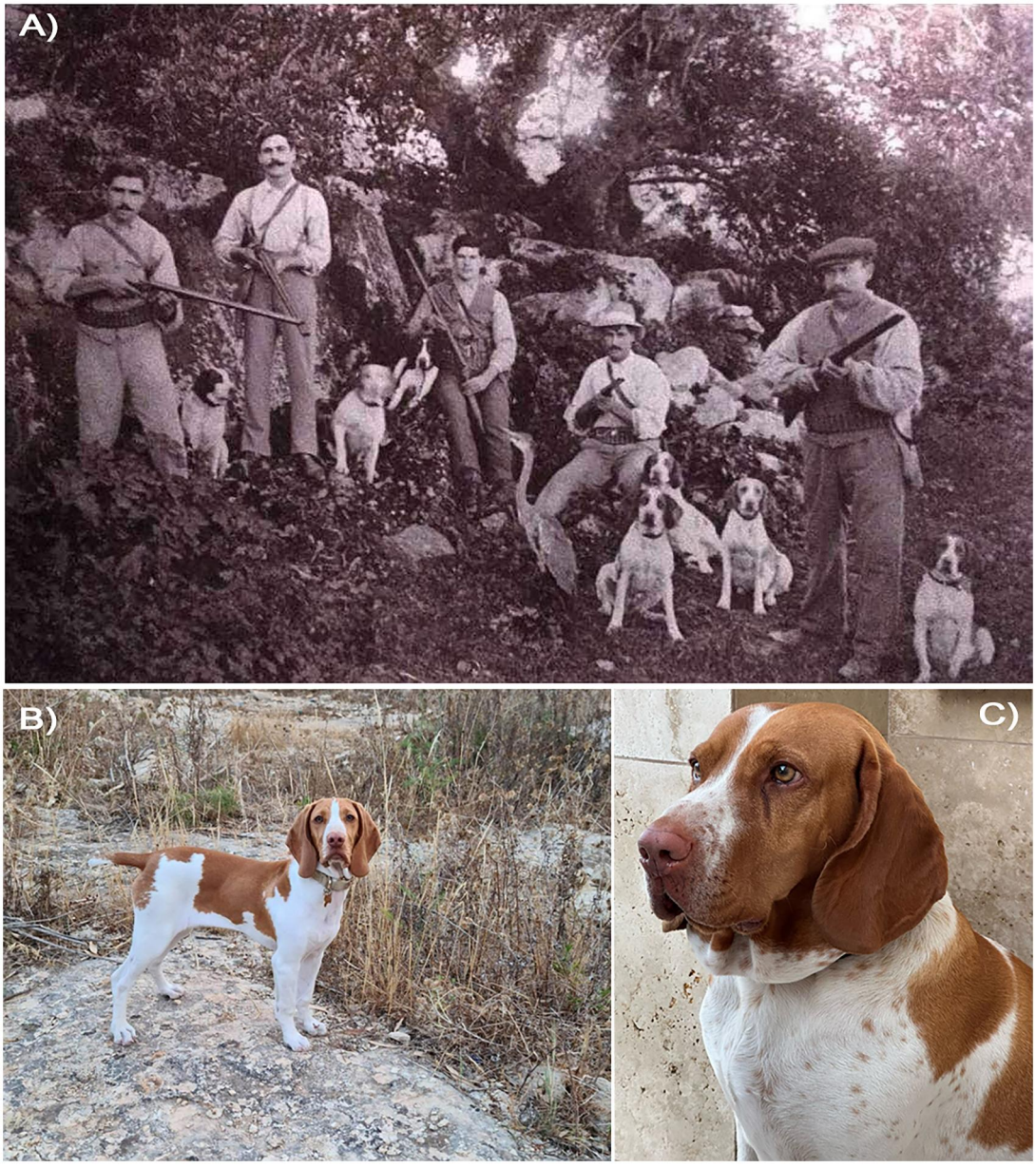

Figure 1. Historical photo featuring maltese hunters with their dogs in the 1930s (unofficial source). The testimonies confirm that it is the maltese hunting dog (A). Recent photo of KTKM specimens: whole body (B) and cranio-facial details (C).

closely related to continental pointing dogs than to Mediterranean sighthounds (Abela 1647).

This canine lineage appears to have been carried forward by what is now referred to as the *Kelb tal-Kaċċa ta' Malta* (Maltese Hunting Dog, KTKM). This dog population shares similar phenotypical traits with other European braque-type dogs, being of medium size with large drop ears and thick hanging lips and having short hair and orange-and-white coat (Figure 1(B/C)). Valued for its versatility in traditional Maltese hunting practices, this dog is used primarily for flushing and retrieving small migratory bird species such as quail and turtle doves. Despite its historical significance (Figure 1(A)), the population has not yet gained official recognition as a breed by the *Fédération Cynologique Internationale* (FCI) nor at the national Maltese level. However, local canine organisations, such as the 'FKNK: Federation for Hunting & Conservation Malta' established on 17 September 1973 (https://www.huntinginmalta.org.mt/), have over the years begun documenting and monitoring the KTKM population. In 2012, the FKNK established the Maltese Hunting Dog Owners' Club and in 2015, 20 KTKM dogs have been studied by Galea and co-workers (2015) suggesting a potential uniqueness of this breed. Afterwards, in 2016, the FKNK together with the Maltese National Canine Federation (MNCF), established a voluntary register in which the breeders can record personal and genealogical information about their

dogs. However, to date, the population structure and the genetic identity of KTKM still remain largely uncharacterised.

In light of its historical and cultural importance, the present study aims to determine whether the population qualifies as a distinct canine breed through phenotypic characterisation and the assessment of its genetic diversity and population structure. Such a designation would justify the adoption of conservation measures to safeguard the KTKM genetic integrity and ensure its long-term viability through the implementation of specifically tailored breeding strategies.

Materials and methods

All breeders participating in this project signed a cooperation agreement. All procedures followed Italian legal regulations (National Directive n. 26/14—Directive 2010/63/UE) and the Association for the Study of Animal Behaviour (ASAB) guidelines for animal treatment in behavioural research and teaching.

Data collection

All measurements and evaluations were conducted between March 2024 and April 2025 by the same commission, composed of two trained individuals. Prior to grading, each commission member received a formal introduction and instructional demonstration from an expert, who acted solely as an instructor and not as a grader in the study. The expert guided the commission members through the procedures of the FCI model standard (https://www.fci.be/en/nomenclature/) for both phenotypic and morphological traits, such as body condition score (BCS). Additionally, the expert assisted handlers in identifying the key anatomical landmarks on dogs to ensure accuracy and appropriateness in the assessments.

Morpho-phenotypic evaluation

A total of 36 (16 males and 20 females) adult *Kelb tal-Kaċċa ta' Malta* (KTKM) were recruited on a voluntary basis to undergo morphological evaluation. To minimise the likelihood of sampling related individuals, dogs were chosen from approximately 30 different households. Additionally, owner-provided information, such as pedigree notes and verbal reports, was used to exclude known first-degree relatives, specifically parent–offspring pairs and full siblings. This was further verified using genomic data by computing pairwise Mendelian error counts as described below. This combined approach ensured that the final dataset consisted of unrelated individuals. The dogs were aged between 1 and 12 years (5.01 ± 2.93, mean ± standard deviation (SD)). The data collected to describe the dogs included the individual animal's identification (date of birth and microchip number), coat characteristics (hair length, texture, colour, and pattern), shape of the tail and ears according to Bonetti (1995), and dental examination (bite, malocclusions and number of teeth) according to Liotta et al. (2024).

Body weight (BW) was recorded using a digital veterinary scale (Foschi, Digital scale for small animals, accuracy: 50 g, max load: 150 kg) and the body condition score (BCS) was visually assessed using a 9–point scale (Laflamme 1997; Christie et al. 2024; Gomes et al. 2025).

The following measures, all expressed in cm, were collected:

- Wither's height (WH) was measured as the vertical distance from the top of the withers to the ground;
- Trunk length (TL) was measured in a straight line along the side of the animal's body, between the point of the shoulder and the point of the ischium;
- Thoracic length (TRL) was measured as the oblique linear distance from the point of the shoulder (cranial angle of the scapula) to the end of the rib cage, at the level of the last rib;
- Chest Circumference (CC) was measured as the girth of the thorax, taken just behind the elbows (armpits of the front legs);
- Total head length (THL) was measured from the occiput (the back of the skull) to the tip of the nose;
- Skull (Cranial) length (SL) was measured from the occiput to the stop;
- Muzzle length (ML) was measured from the stop to the tip of the nose;
- Bizygomatic distance (BD) was measured as the distance at the widest part of the head, between the zygomatic arches, excluding the ears, by placing the dog looking straight forward.

Linear body measurements, such as withers height, trunk length, and thoracic length were recorded using a measuring stick. The circumference was determined with a standard measuring tape, while the dimensions of the head were taken using a precision calliper.

Based on these body and head measurements, two morphological ratio indices were estimated following protocols established by Bonetti (1995):

- Cephalic Index (CI): Bizygomatic distance (BD) x 100/Total Head Length (THL);

- Body Index (BI): Trunk Length (TL) x 100/Chest Circumference (CC).

The descriptive statistics of the morphometric values, such as mean, standard deviation (SD) and the quartiles (first quartile, median, third quartile), were obtained using the base function implemented in the stats R package. Moreover, skewness and kurtosis were calculated using the *e1071* and *moments* R packages. The normality of variable distributions was assessed using the Shapiro-Wilk test (shapiro.test, from the *stats* R package). To test for a sex effect between the two genders, a t-test was performed using the *stats* package in R. BW and CC measurements of dogs with BCS equal or less than 3/9 or equal or greater than 7/9 were excluded, to minimise the influence of under- or over-conditioning, as excessive fat or muscle loss can distort weight and chest circumference measurements independent of skeletal size.

Genomic analyses - dataset and quality check

Biological samples were collected from 34 unrelated KTKM. DNA extraction and genotyping with Illumina Canine 230K SNP chips were performed through an outsourced laboratory. The dataset was compared with publicly available SNP data from hunting dog breeds (pointers, hounds, spaniels), primitive breeds such as Cirneco dell'Etna (CIRN) and the Maltese *Kelb tal-Fenek* (PHAR), terriers, and shepherd dogs (Parker et al. 2010; Talenti et al. 2018) (Table 1). These breeds were included to define the genetic structure, uniqueness, and phylogenetic relationships of the Maltese hunting dog. The hunting dog group was specifically included to facilitate the understanding of the functional clusters and phenotypic similarity; the primitives were aimed at exploring possible links due to shared cradle of origin; and the terriers and the shepherd breeds were incorporated to detect possible genetic

Table 1. Sample information and breeds' diversity statistics.

Breed code	Breed name	Functional Group	Origin *N* (QC)	Ho	He	F_{ROH} (SD)
KTKM	Kelb tal-Kaċċa ta' Malta	–	34 (33)	0.32	0.32	0.11 (0.05)
BASS	Basset Hound	Hunting Hounds	10 (10)	0.25	0.24	0.30 (0.06)
BEAG	Beagle	Hunting Hounds	10 (10)	0.28	0.28	0.22 (0.04)
DALM	Dalmatian	Hunting Hounds	9 (9)	0.28	0.28	0.20 (0.03)
FOXH	Fox Hound	Hunting Hounds	10 (10)	0.31	0.31	0.11 (0.06)
OTTR	Otter Hound	Hunting Hounds	9 (9)	0.23	0.25	0.27 (0.06)
REDB	Red Bone Coonhound	Hunting Hounds	2 (2)	0.25	0.3	0.18 (0.16)
SIPF	Segugio Italiano Pelo Forte	Hunting Hounds	16 (16)	0.34	0.34	0.07 (0.04)
SIPR	Segugio Italiano Pelo Raso	Hunting Pointers	16 (16)	0.33	0.33	0.09 (0.06)
BRAC	Bracco Italiano	Hunting Pointers	12 (11)	0.27	0.28	0.22 (0.03)
BRIT	Brittany	Hunting Pointers	10 (10)	0.3	0.29	0.19 (0.08)
ESET	English Setter	Hunting Pointers	15 (14)	0.28	0.28	0.19 (0.05)
GORD	Gordon Setter	Hunting Pointers	9 (9)	0.3	0.3	0.17 (0.08)
GSHP	German Shorthaired Pointer	Hunting Pointers	10 (10)	0.31	0.31	0.13 (0.06)
GWHP	German Wirehaired Pointer	Hunting Pointers	2 (2)	0.25	0.31	0.14 (0.06)
ISET	Irish Setter	Hunting Pointers	9 (9)	0.25	0.26	0.25 (0.05)
KMUN	Large Munsterlander	Hunting Pointers	3 (3)	0.25	0.3	0.17 (0.03)
SPIN	Spinone Italiano	Hunting Pointers	23 (22)	0.32	0.32	0.12 (0.05)
VIZS	Vizsla	Hunting Pointers	7 (7)	0.28	0.3	0.17 (0.03)
WEIM	Weimaraner	Hunting Pointers	10 (10)	0.24	0.24	0.31 (0.07)
WHPG	Wirehaired Pointing Griffon	Hunting Pointers	6 (6)	0.28	0.31	0.15 (0.05)
ACKR	American Cocker Spaniel	Hunting Spaniels	10 (10)	0.24	0.24	0.32 (0.05)
ECKR	English Cocker Spaniel	Hunting Spaniels	9 (10)	0.27	0.26	0.25 (0.09)
ESSP	English Springer Spaniel	Hunting Spaniels	10 (10)	0.26	0.26	0.28 (0.14)
FIEL	Field Spaniel	Hunting Spaniels	4 (4)	0.2	0.23	0.36 (0.09)
IWSP	Irish Water Spaniel	Hunting Spaniels	10 (10)	0.27	0.28	0.23 (0.06)
LAGO	Lagotto Romagnolo	Hunting Spaniels	23 (22)	0.31	0.31	0.16 (0.05)
PTWD	Portuguese Water Dog	Hunting Spaniels	10 (10)	0.28	0.28	0.25 (0.07)
CIRN	Cirneco dell'Etna	Primitive	20 (20)	0.32	0.3	0.17 (0.13)
PHAR	Pharaoh Hound	Primitive	17 (17)	0.28	0.27	0.24 (0.06)
MANN	Mannara Dog	Shepherd	12 (22)	0.33	0.32	0.13 (0.08)
MARM	Maremma Sheepdog	Shepherd	16 (14)	0.32	0.33	0.10 (0.06)
AUCD	Australian Cattle Dog	Shepherd	10 (10)	0.3	0.31	0.15 (0.05)
AUSS	Australian Shepherd	Shepherd	10 (10)	0.3	0.3	0.19 (0.06)
BMAL	Belgian Malinois	Shepherd	6 (6)	0.3	0.32	0.13 (0.05)
BELS	Belgian Sheepdog	Shepherd	10 (10)	0.26	0.27	0.26 (0.03)
BERG	Bergamasco	Shepherd	11 (10)	0.3	0.32	0.15 (0.09)
BORD	Border Collie	Shepherd	10 (10)	0.3	0.31	0.14 (0.05)
OES	Old English Sheepdog	Shepherd	10 (10)	0.27	0.27	0.24 (0.05)
TYFX	Toy Fox Terrier	Terriers	4 (4)	0.28	0.3	0.18 (0.12)
WFOX	Wire Fox Terrier	Terriers	10 (10)	0.22	0.23	0.35 (0.07)
APWF	Appenine Wolf	Wild	8 (8)	–	–	–

N and QC: Sample size before (N) and after (QC) quality check; Ho: Observed heterozygosity; He: Expected heterozygosity; F_{ROH}: Runs of homozygosity-based inbreeding; SD: standard deviation.

introgressions. Quality control filtering was performed using PLINK v1.9 (Chang et al. 2015). Individuals with a call rate below 94% were excluded, and SNPs were removed if they were unlocated, located on sex chromosomes, had a call rate below 95%, or had a minor allele frequency (MAF) below 1%. To minimise bias due to close kinship, highly related individuals were detected among the pairs with less than 100 Mendelian errors and excluded from this analysis. Mendelian errors were computed for every pairwise combination, treating each pair as a putative parent–offspring pair. Subsequently, we excluded the minimum number of animals necessary to eliminate all pairs showing direct relatedness, thereby ensuring that no first-degree relatives were retained in the dataset.

Genomic analyses - population structure and genomic background

To investigate the population structure and genetic distinctiveness of the KTKM, multidimensional scaling (MDS) analysis was conducted using PLINK with the *–cluster* flag.

In addition, to investigate broad patterns of genetic variation, PHATE (Potential of Heat-diffusion for Affinity-based Transition Embedding) was applied to the first 20 principal coordinates obtained from MDS, using the phateR v1.0.7 library (Moon et al. 2019). This method reduces complex high-dimensional data through a diffusion process, producing a low-dimensional embedding that captures both local structure and global relationships, and is particularly effective at revealing continuous genetic trajectories or branching patterns. PHATE was previously applied to canine genetic data by Dutrow et al. (2022) with successful results; therefore, we used the same parameter settings to generate a 2-dimensional PHATE: knn = 80, gamma = 0, and decay = 100.

Reynolds genetic distances among all breeds were calculated with an in-house script, while individual identity-by-state (IBS) distances were generated using PHYLIP (Felsenstein 1989). Genetic distances were bootstrapped 100 times, and the resulting phylogenetic trees were visualised using the *ggtree* R package (Xu et al. 2022).

To assess historical gene flows, Treemix v1.13 (Pickrell and Pritchard 2012) analysis was performed, testing models ranging from 0 to 15 inferred migration events, using the Apennine Wolf as outgroup. The optimal number of migration edges was determined based on the fraction of variance explained. Additionally, the *f3* test provided within Treemix was used to detect potential gene flow events between populations.

The genetic ancestry of individuals was further examined using ADMIXTURE v1.3 (Alexander et al. 2009), testing K values from 2 to 34. The optimal K was determined based on the lowest five-fold cross-validation error. Haplotype sharing patterns were analysed using RefinedIBD v4.1 (Browning and Browning 2013), applying a sliding window size of 1 Mb, a trimming length of 0.15 Mb, and retaining segments with a minimum length of 0.2 Mb and LOD score of 3.0. The median total length of shared segments was calculated across individual comparisons within each breed pair, assigning a value of 0 to pairs with no shared haplotypes. The top 5% of haplotype sharing was visualised using the *circlize* R package (Gu et al. 2014).

Genomic analyses - genomic diversity

Genomic diversity metrics, including observed (*Ho*) and expected (*He*) heterozygosity, were calculated in PLINK. Runs of homozygosity (ROH) were detected using a sliding window approach in PLINK. The minimum number of SNPs to call a ROH was calculated using the Lencz et al.'s formula (Lencz et al. 2007; Purfield et al. 2012; McQuillan et al., 2020), resulting in a value of 59; the same value was applied to define the window size. The minimum ROH length threshold was set to 1 Mb, with a gap of 1 Mb. No heterozygous SNPs and a maximum of 5 missing SNPs were allowed. Maximum inverse density was set to 24 kb/SNP after calculating ROH coverage with density ranging from 19 (equal to the mean density of the analysed markers) to 250 kb/SNP (McQuillan et al. 2020). ROH coverage was equal to 98.8% using these parameters. The inbreeding coefficient (F_{ROH}) was computed following the McQuillan formula (McQuillan et al. 2008) considering different ROH length classes (1–2 Mb, 2–4 Mb, 4–8 Mb, 8–16 Mb, and >16 Mb) to evaluate inbreeding levels across different timescales.

Finally, effective population size (*Ne*) of the two Maltese dog breeds - KTKM and PHAR- was estimated through GONE v1.0 (Coombs et al. 2012) using default parameters, and SNeP v1.11 (Barbato et al. 2015) using Sved & Feldman's mutation rate modifier (Sved and Feldman 1973) sample size correction and a permitted maximum distance between SNP pairs of 200 Mb.

Results

Morpho-phenotypic evaluation

The 36 KTKM (16 males and 20 females) enrolled in this study had an average BCS of approximately

Table 2. Descriptive statistics of morphological traits and indices for male and female KTKM.

	Sex	Mean	SD	Q1	Median	Q3	Skewness	Kurtosis	Shapiro-Wilk (*p-value*)[b]	*T*-test DF (*p*-value)[c]
Withers height (WH)	M	46.7	2.3	45.2	46.7	48.8	−0.28	1.92	0.94 (0.36)	33.85 (0.001)
	F	43.2	3.1	41.5	43.8	44.5	0.42	3.65	0.96 (0.51)	
Trunk length (TL)	M	58.2	3.3	56.3	57.7	60.3	−0.49	3.49	0.96 (0.62)	30.28 (0.001)
	F	54.3	2.9	53.1	54.7	56.4	−0.52	3.14	0.95 (0.38)	
Thoracic length (TRL)	M	34.9	2.2	33.9	35.2	36.1	−0.04	2.63	0.94 (0.37)	27.85 (0.17)
	F	33.3	4.7	29.7	33.1	34.8	1.64	6.96	0.84 (0.001)	
Chest Circumference (CC)[a]	M	67.9	7.1	64.6	68.3	73.8	−0.49	2.65	0.97 (0.70)	26.67 (0.40)
	F	66.0	5.2	61.0	66.2	70.2	0.10	1.57	0.91 (0.04)	
Total head length (THL)	M	20.5	1.4	19.0	21.0	21.8	−0.10	1.27	0.84 (0.008)	27.37 (0.001)
	F	18.4	1.1	18.0	18.3	19.0	0.35	2.92	0.96 (0.44)	
Skull length (SL)	M	13.1	1.3	12.2	13.1	14.0	−0.76	2.89	0.91 (0.13)	26.87 (0.05)
	F	12.2	0.9	11.7	12.2	13.0	−0.22	2.86	0.98 (0.95)	
Muzzle length (ML)	M	7.6	1.3	6.9	7.9	8.0	0.24	3.02	0.97 (0.82)	31.30 (0.03)
	F	6.7	1.2	5.9	6.6	7.0	1.03	4.30	0.92 (0.11)	
Bizygomatic distance (BD)	M	10.9	0.8	10.0	11.0	11.1	0.81	3.46	0.88 (0.02)	21.86 (0.001)
	F	10.2	0.5	10.0	10.0	10.3	0.86	3.85	0.86 (0.001)	
Cephalic index (CI)	M	53.4	4.9	49.3	52.6	57.9	−0.04	1.93	0.96 (0.55)	28.31 (0.21)
	F	55.5	4.6	53.1	55.4	58.1	0.13	2.76	0.98 (0.87)	
Body Index (BI)	M	86.4	8.5	80.8	87.3	90.7	1.01	4.08	0.95 (0.44)	27.41 (0.15)
	F	82.6	6.4	77.8	80.4	87.8	0.39	2.29	0.93 (0.16)	

All measurements are expressed in cm, with the exception of indices.
[a]Only measures of subjects with BCS between 4 and 6 were included.
[b]Shapiro-Wilk test to assess if data were normally distributed. The test compares the scores in the samples to a normally distributed set of scores with the same mean and standard deviation; $p < 0.05$ indicates that variable is not normally distributed.
[c]t-test considering M vs F.

5.69 ± 1.51 (mean ± SD). The recorded body weight was 23.43 ± 6.98 (mean ± SD) for males and 21.25 ± 3.45 (mean ± SD) for females, excluding dogs with BCS equal or less than 3/9 or equal or greater than 7/9.

All animals had a short, dense, and smooth coat. The most common colour pattern was piebald, with orange markings ranging from lemon (a pale, diluted yellow-orange) to a deep reddish-fawn (Figure 1(B)). More than one-quarter of dogs (n. 10) also presented a ticked pattern (Figure 1(C)). The skin was firm, with mild muscular definition at rest. Nose pigmentation ranged from pink to flesh-coloured, and the eyelid rims were pink, while the eyes were amber (Figure 1(C)). In 83.3% of individuals, the tail was docked (Figure 1(B)), with 50% retaining approximately one-third of the potential natural length and 33.3% retaining two-thirds to three-quarters. The remaining 16.6% had natural tails, well set, not deviated, and slightly curved at the last third. No cases of natural bobtail conditions were observed.

Regarding dentition, 88.6 of dogs showed a scissor bite, considered as standard for functional efficiency, 8.6% (3/35) had a level bite, and 2.9% (1/35) showed minor malocclusions or prognathism; no cases of severe prognathism, enognathism, or cross-bite were recorded.% (31/35).

Limb deviations, particularly cow hocks (medial deviation of the hocks) and outward deviation of the pasterns, were observed among the examined individuals, mainly affecting dogs with a BCS equal or greater than 7/9 and approximately 10 years of age.

According to the evaluation of morphological traits (Table S1), males and females showed some different measurements, with statistically significant differences in WH, TL, SL, ML, and BD (Table 2). Additionally, WH, TL, and TRL in males, THL in females, as well as SL, ML, CI, and BI, were normally distributed according to the Shapiro-Wilk test ($p > 0.05$; Table 2). Although the population exhibited a reasonable degree of morphological consistency, TRL and CC in females, THL in males, and BD in both sexes did not follow a normal distribution ($p < 0.05$; Table 2).

A certain grade of sexual dimorphism was observed, with males and females showing different size and proportions as evidenced by body index (BI) estimation. According to the BI ranges (<85 dolichomorphic; 71–84 mesomorphic; <70 brachymorphic; (Bonetti 1995), males can be defined as slightly dolichomorphic (86.39; Table 2) while females are mesomorphic (82.61; Table 2).

According to the cephalic index (CI) both males and females can be classified as mesocephalic following the description given by Liotta et al. (2024) (CI < 55: dolicocephalic type; CI = 50–55: mesocephalic type; CI > 50: brachycephalic type).

Genomic analyses

The final dataset following quality control included 111,155 SNPs and 457 dogs across 41 breeds. Additionally, 8 Apennine wolves (APWF) were used as outgroups in some of the analyses. Genetic diversity was assessed across all breeds using heterozygosity measures and F_{ROH} estimates (Table 1). The KTKM

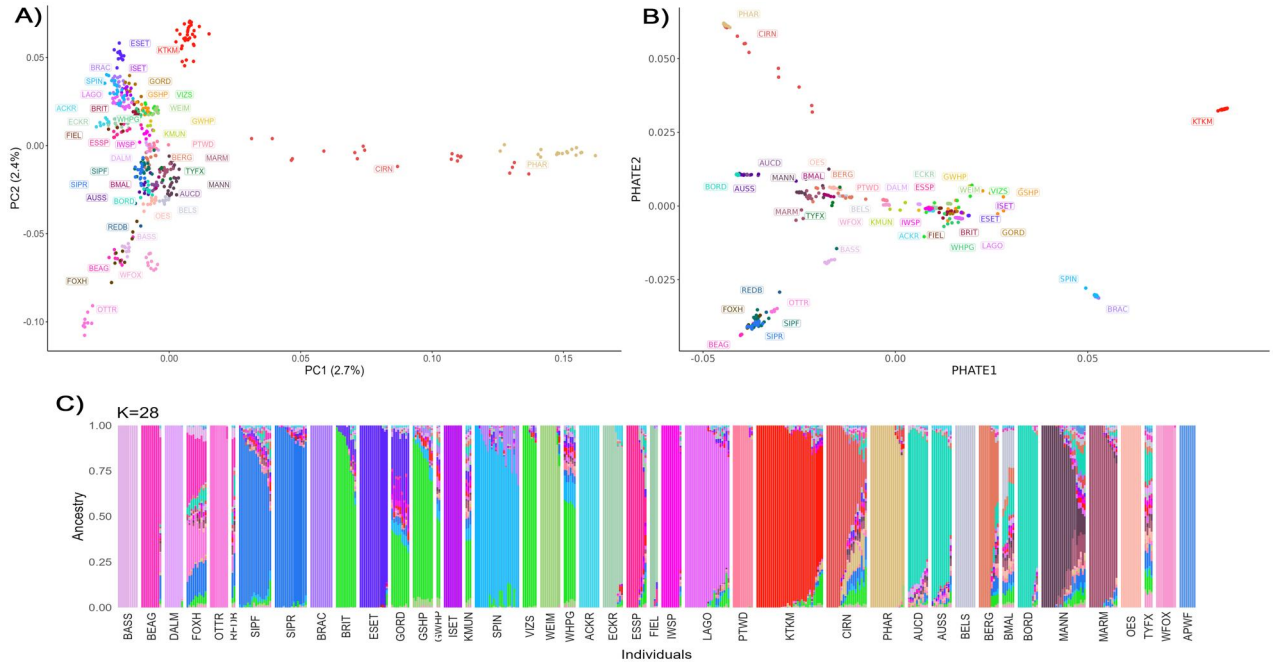

Figure 2. Scatterplot of the first two principal components (PCs) of multidimensional scaling (MDS, A) and of PHATE (B), and individual admixture of the analysed breeds (C). The admixture model with the lowest cv error was found with a number of clusters (K) equal to 28. In the admixture plot, breeds are ordered according to the functional group they belong to.

exhibited a good level of heterozygosity, with an observed value of 0.324, slightly exceeding the expected value of 0.321. Additionally, with an average F_{ROH} of 11.06 ± 5.33% the KTKM ranked with those breeds having the lowest inbreeding coefficients. The distribution of F_{ROH} across different ROH length classes indicated a balanced contribution of both ancient and recent inbreeding (Figure S1). Notably, the proportion associated with recent inbreeding was slightly higher, possibly reflecting the impact of recent, more structured selection practices and the introduction of voluntary pedigree registration. However, this proportion remained comparable to, or lower than, what is observed in most other breeds. The genomic Ne calculated at the most recent generation, for KTKM and PHAR respectively, was equal to 62 and 112 according to GONE and 31 and 19 according to SNeP. Specifically, results from GONE software showed a decreasing trend spanning 74 to 27 generations ago, followed by a second drop occurring 12 generations ago. On the other hand, the PHAR trend showed a drop earlier than KTKM, about 39 to 17 generations ago, followed by a stable trend (Figure S2).

Multidimensional scaling (MDS) clustered breeds according to their functional groups. Specifically, the two primitive breeds, the Cirneco dell'Etna (CIRN) from Sicily (Italy) and the Maltese PHAR were separated along PC1, whereas the other breeds could be grouped according to their functional group along PC2, from top to bottom: pointing dogs, spaniels, terriers, shepherds, and hounds (Figure 2(A)). Notably, the KTKM group formed a well-defined cluster, positioned near, but clearly isolated along both the first two PCs, the group of pointing hunting dogs, consistent with their functional selection history. Interestingly, they were slightly displaced along PC1, trending towards the primitive breeds, likely reflecting geographic and historical connections. In the PHATE plot (Figure 2(B)), the functional and geographic distinction of the analysed dogs became even more clear, as well as the isolation of the KTKM from the other breeds.

The ADMIXTURE analysis (Figure 2(C) and Figure S3) demonstrated that the KTKM formed a breed-specific cluster at relatively low K values. Prior to this differentiation, at $K = 4$ (Figure S4), all individuals shared a similar background, primarily composed of the cluster common to the pointing dog and spaniels. Furthermore, a notable proportion of ancestry associated with both the primitive dogs and, in some individuals, to the hounds was evident in the breed. According to the cross-validation errors (cv), the best-fitting model was found at $K = 28$ (Figure 2(C)). In this scenario, KTKM individuals exhibited nearly exclusive membership to a unique cluster, which was not substantially shared with any other breed. Only a limited number of KTKM exhibited significant proportions of the background common to a cluster not specifically associated to any breed, but widely shared by different pointing dogs, such as Brittany, German

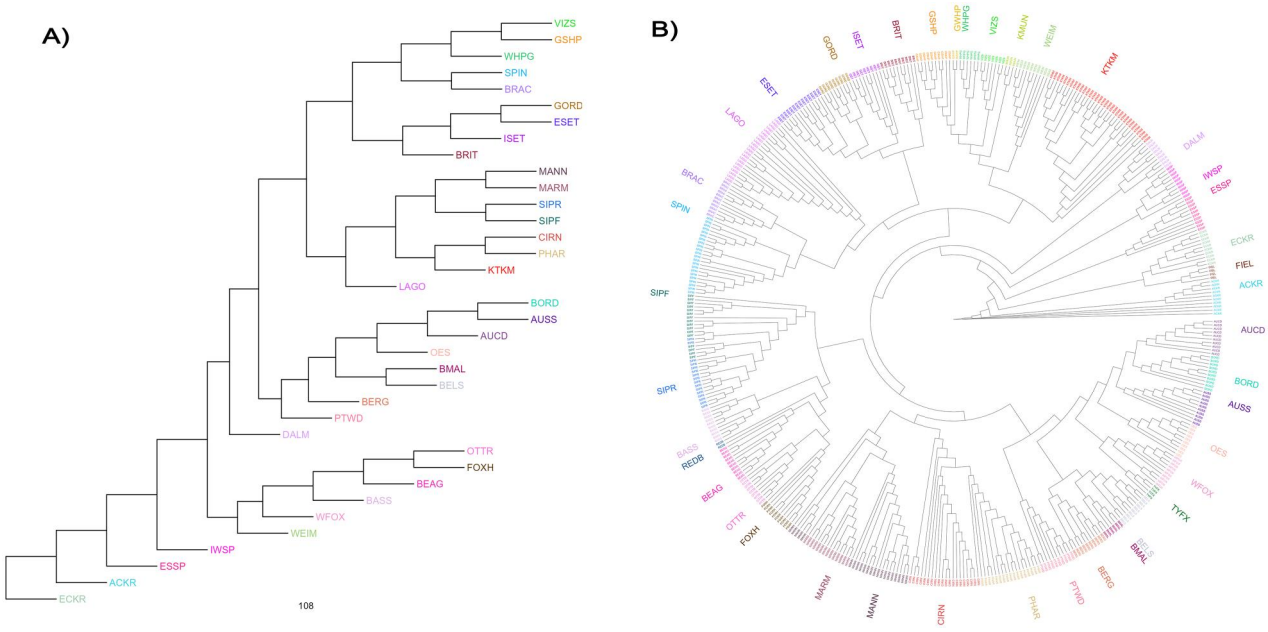

Figure 3. Dendrograms of Reynolds genetic distances among populations (A) and individual identity-by-state (IBS) distances among individuals (B).

Shorthaired Pointer, and Vizsla (BRIT, GSHP, and VIZS, respectively). Interestingly, this level of genetic distinction was not observed in several long-established and widely recognised breeds, particularly within the herding group and some pointer breeds, which exhibited substantial cluster sharing.

The dendrogram constructed using Reynolds distances at the population level revealed four primary branches corresponding to pointing dogs, Maltese and Italian dogs, shepherd dogs, and hounds, whereas spaniels were located at the base of the tree (Figure 3(A)). The KTKM clustered within the second branch, alongside the CIRN and the PHAR, but also showed proximity to the pointing dogs. In contrast, the individual-level IBS-based tree (Figure 3(B)) placed the KTKM at the end of the branch comprising the pointing dogs. Importantly, all individuals, including KTKM, clustered tightly within their respective breeds, with no evidence of recent admixture or interchange between breeds.

Four was identified as the most informative number of migration edges using TreeMix (Figure 4(A)). In this model, KTKM was located at the base of the branch grouping the ointing dogs. One of the identified gene flows linked the PHAR to KTKM; other migration events were found between two setter breeds, specifically from Gordon Setter (GORD) to Irish Setter (ISET), between the American (ACKR) and English (ECKR) Cocker Spaniels, and from common node of Australian Cattle Dog (AUCD), Border Collie (BORD), and Australian Shepherd (AUSS) directed towards the branch common to the hounds (Figure 4(A)).

The IBD-based haplotype sharing analysis revealed significant sharing primarily among breeds within the same functional group (Figure 4(B)). Notably, the Field Spaniel (FIEL)–ECKR pair exhibited the highest levels of haplotype sharing. When focusing on the top 5% of shared haplotypes, the KTKM did not show significant sharing with any other breed. Nevertheless, its highest levels of haplotype sharing were observed with the GSHP (27.2 Mb), followed by PHAR (26.5 Mb), German Wirehaired Pointer (GWHP, 21.3 Mb), and ECKR (17.3 Mb) breeds.

Discussion

The concept of a breed is a subject of considerable debate. According to European law, specifically Regulation (EU) 2016/1012, an animal population can be defined as a breed if it exhibits sufficient phenotypic uniformity to allow breeders to distinguish it from other individuals of the same species, and if a breeding book recording genealogical information has been established. While this legal definition focuses on morphological homogeneity, other factors such as a population's historical and cultural significance, its history of local selection, and its genomic distinctiveness are widely recognised as complementary criteria for breed recognition. Phenotypic and genomic analyses are therefore essential for identifying and conserving animal genetic resources (Sponenberg et al. 2018), especially for locally important but unofficially recognised populations (Gandini and Villa 2003; Hoffmann

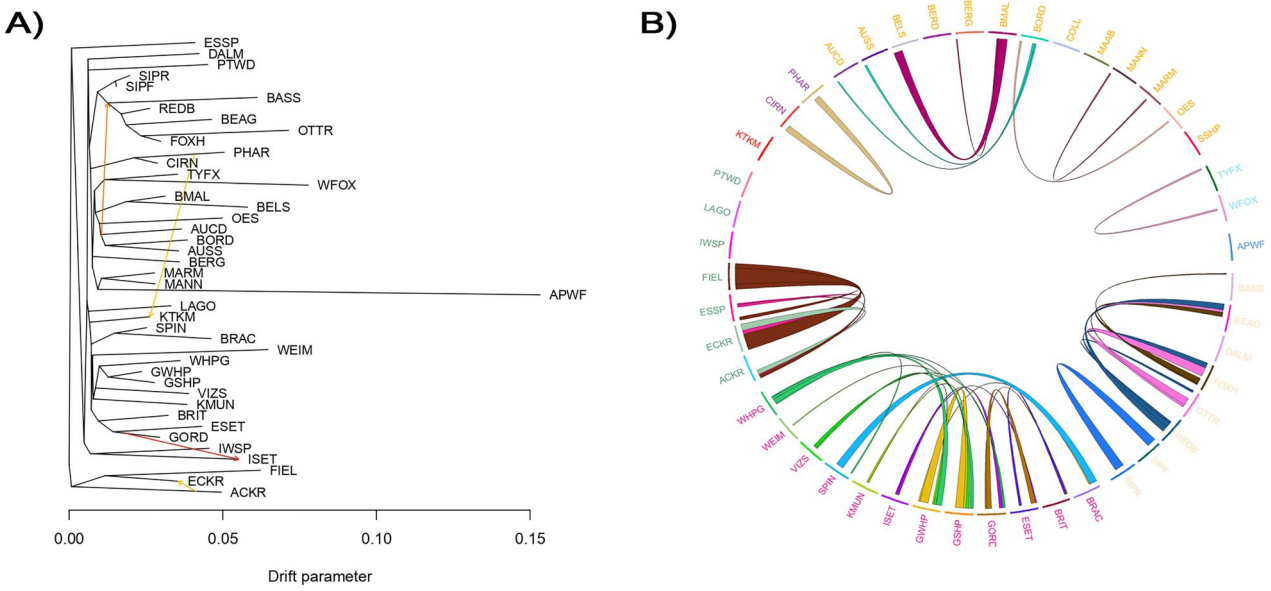

Figure 4. TreeMix dendrogram with 4 migration events (A) and IBD-based haplotype sharing among breeds (B). Only 5% longest shared segments are reported. The width of the arches connecting breeds are proportional to the median of the total length of the shared segments. Text colour indicates the breed functional group.

2013; Di Trana et al. 2015; Szpiech et al. 2021). This is the case for the *Kelb tal-Kaċċa ta' Malta* (KTKM), a canine population long known and used by Maltese hunters, yet never formally recognised as a breed. Its development and selection have therefore relied exclusively on the practices and preferences of local breeders and hunters, which shaped a dog known for its friendly and intelligent temperament and displaying a strong aptitude for flushing, retrieving, and other hunting activities.

Morpho-phenotypic evaluation

The morpho-phenotypic description is a fundamental step to assess visible uniformity of the individuals belonging to a population and describe the typical traits of a breed, which can pose the basis for drafting a breed standard that, in accordance with FCI guidelines (https://www.fci.be/en/nomenclature/), will be fundamental for the future official recognition of the breed.

The most commonly observed KTKM coat colour is piebald with orange markings ranging from lemon to fawn; some dogs ($n = 10$, 27.7%) showed ticking on white areas, a trait that, when excessive, is considered by breeders as tolerated but not desirable.

The shape of the skull is one of the most important criteria for determining standard dog breeds and, according to CI, KTKM can be classified as mesocephalic. In fact, the head was observed to be well-proportioned, with a moderately rounded skull and parallel or only slightly diverging craniofacial axes. The eyes are usually in ochre colour, medium-sized, almond-shaped, and slightly obliquely set, which contributes to a wide lateral field of vision, typical of braque-type dogs (Evans and Lahunta 2013). The ears are set slightly above the eye line and carried pendulously. The nose is large and well developed, with open nostrils and a slight protrusion beyond the lips, forming a distinct angle and its pigmentation ranges from pink to flesh-coloured.

In terms of dentition, 88.6% of examined dogs exhibited a scissor bite, 8.6% presented a level bite, while 2.9% displayed minor malocclusions or mild prognathism. The observation of a relatively high proportion of dogs with a level bite could justify an initial tolerance for this occlusion during the early stages of breed recognition, while still encouraging breeders to favour the scissor bite, as it is considered the functional standard (Liotta et al. 2024).

With regard to tail, a high proportion of the examined dogs appeared to be docked, a practice likely regarded as a preventive measure against tail injuries during hunting by their owners. No naturally brachyure or anure individuals were observed. In undocked dogs, the tail was well set, in line with the croup, straight, and only slightly curved towards the end. When the dogs are excited, the tail may be carried more upright, though it should never curl over the back.

The BCS is a widely used indicator of proper nutrition and health, although not always reflecting the

true status of working dogs. Considering a scale ranging from 1 to 9, dogs within a 1–3 range are considered to be too skinny, the score from 4 to 5 is considered as being ideal, while a score from 6 to 9 is associated with overweight individuals and generally not recommended (Laflamme 1997). Most of the enrolled KTKM showed the ideal values of BCS, allowing them to correctly take measurements to describe the KTKM.

Phenotypical data revealed a good level of morphological uniformity across the population, although some degree of variability emerged. The observed variability is consistent with the range of phenotypic variation previously reported in other recognised canine breeds (Sutter et al. 2008).

The evaluation of conformation indices indicates the presence of sexual dimorphism, as often observed in the domestic dog species (Sutter et al. 2008). In fact, males and females showed different sizes and proportions, according to the body index (BI) classification. Specifically, male KTKM can be defined as slightly dolichomorphic (86.4), while females are mesomorphic (82.6). In general, BI can be linked to the functional capabilities of animals: BI above 85 (dolichomorphic type) are typically associated with long and fast animals; BI between 71 and 84 (mesomorphic type) describe animals with medium proportions and greater strength; while BI below 70 (brachymorphic type) define short animals (Bonetti, 1995). KTKM, as described by hunters, are both fast and strong with a balanced, robust, and functional build ideal for working on rustic environments. In general, the KTKM conveys an image of a hardy, athletic, and well-proportioned dog, capable of performing in demanding conditions while maintaining a solid and balanced morphology. All of these considerations are supported by the average body weight and proportions which classify KTKM in a medium to large size class, according to the classification proposed by Salt et al. (2017).

In general, the topline of these dogs has a gentle upward slope, giving the dog a natural, slightly rising profile. They also have a deep, broad chest that supports strong respiratory capacity, and a compact, muscular build that reflects stamina and strength. Both the forelimbs and hindquarters show good bone structure and muscular development, contributing to efficient movement and physical endurance. Limb deviations were consistently observed in a significant portion of the sample, including cow hocks (medial deviation of the hocks) and outward deviation of the patterns. While these traits do not necessarily impair basic function, they are considered faults under most functional and structural standards and should be monitored in future breeding programs aimed at preserving health and efficiency. Morphometric traits characterising the KTKM such as medium muscular build, strong limbs, long muzzle and ears are what breeders and local hunters consider as assets for scenting, flushing, pointing, and endurance in local field conditions.

Genomic analyses

Although not mandatory for official breed recognition, genomic analyses offer valuable insights to assess whether an animal population meets the criteria of a breed. They also help reconstruct its origin and evolutionary history, identify unique genetic features, detect admixture or inbreeding, and provide tools to monitor genetic diversity and optimise breeding management over time (Toro and Caballero 2005; Sponenberg et al. 2018).

To better understand the history of KTKM and assess its distinctiveness, we included a range of reference breeds in our analyses, selected based on functional and morphological similarities (i.e. pointer- and spaniel-type dogs); shared morphological traits such as coat colouration, ear shape, and general expression (i.e. hounds); anecdotal reports of possible introgression (i.e. terriers and shepherd breeds); and the hypothesised historical connection to primitive breeds such as the *Kelb tal-Fenek* (PHAR), long used in Maltese hunting contexts.

The KTKM placement relative to other breeds, on one side confirmed the known history of the population, and on the other the possible shared ancestry with hunting dogs with similar phenotype and/or function, depending on the genetic patterns captured by the different analyses. Indeed, phylogenetic reconstructions based on Reynolds' genetic distances allow to explore more ancient or population-level relationships, reflecting long-term genetic differentiation driven by drift and divergence (Reynolds et al. 1983), whereas IBS-based trees emphasise recent or individual-level relationships, reflecting more contemporary shared ancestry or recent gene flow (Powell et al. 2010). Therefore, it is not surprising that the first method revealed an ancient connection between the KTKM and the other breed originated in Malta, the PHAR, whereas more recent relationships were found with continental pointer and, to a lesser extent, spaniel breeds, similar in morphology and hunting style and likely introduced into the Maltese archipelago in later times. Such historical mixing likely contributed to

the functional traits observed today. In the case of the KTKM, pointer ancestry likely underpins pointing ability, scenting, and endurance; spaniel input contributes versatility and retrieval; while PHAR influence supports local adaptation and continuity with regional hunting traditions. The hypothesis is that the combination of these traits, together with features shaped by local adaptation, produced a phenotype functionally optimised for bird hunting in the Maltese territorial environment.

Despite this complex genomic background, all analyses consistently highlight the KTKM as a population that is highly distinct from other breeds while maintaining remarkable genetic uniformity. Dimensionality reduction approaches such as PHATE and MDS, indeed, cluster KTKM individuals tightly, IBS-based tree places all individuals within the same clade, and admixture profiles reveal a largely uniform genome with only minor traces of other breed contributions. In fact, the separation of KTKM from other dogs appears more pronounced than that observed for many recognised breeds, consistent with findings in other studies of Italian and Mediterranean dog populations (Talenti et al. 2018). Importantly, this genetic 'isolation' does not appear to result from excessive inbreeding or the repeated use of closely related individuals. Within our sample, measures of homozygosity and heterozygosity suggest moderate genetic variability, with inbreeding levels relatively low, especially if compared with other recognised breeds.

The effective population size has been estimated for the two most important Maltese dog breeds, KTKM and PHAR. These dogs share several similarities, such as a long history of local development within the same geographic area and, consequently, the effect of insularity, small population sizes, and use as hunting dogs. However, their recognition histories are different, making it particularly interesting to evaluate how demographic history determines differences in effective Ne values in two relatively similar populations (Leroy et al. 2013). Although we observed differences in the estimated Ne, the values are comparable to that reported for other recognised dog breeds, calculated with similar methods (Dreger et al. 2016). The discrepancy in Ne values obtained with different softwares is likely due to the fact that the GONE software relies on temporal evolution of linkage disequilibrium (LD) across generations, providing insights into changes in Ne over time (Coombs et al. 2012), whereas SNeP estimates Ne from the decay of LD with genetic distance at a single time point and therefore tends to provide a more 'averaged' or smoothed view of effective size in recent generations (Barbato et al. 2015). Therefore, the two methods rely on different assumptions and, for example, GONE is often more sensitive to historical fluctuations and bottlenecks (Coombs et al. 2012), while SNeP provides estimates that reflect more contemporary patterns of genetic drift (Barbato et al. 2015). In light of this and given the importance for conservation purposes of this parameter and to facilitate comparison with other studies, we implemented both methods to calculate Ne, as already suggested by Kidner et al. (2021) and Becker et al. (2024). In conclusion, Ne estimated for KTKM reflects an effective, though not structured, management of the population by breeders and hunters, who pursued a functional and morphological selection while retaining a broad genetic base.

The results obtained from genomic analyses, further corroborated by phenotypic data, strongly support the recognition of the KTKM as a distinct breed, likely belonging to the pointing group, albeit demonstrating high versatility during hunting practice.

Limitations and future perspectives

The present study constitutes the first comprehensive effort to describe the KTKM breed at both phenotypic and genomic levels, thereby laying the foundation for its official recognition. For this type of characterisation, it is essential to obtain data from a representative sample of the target population, encompassing unrelated individuals that can be confidently regarded as members of the breed, while simultaneously preserving the existing genetic variability (Sponenberg et al. 2018). In the present study, 36 dogs were analysed. Sampling was based on phenotypic appearance and, when available, genealogical information provided by the owners; moreover, particular attention was paid to minimising relatedness and to include dogs from different owners. Nevertheless, the limited population size and the voluntary nature of owner participation constrained the collection of additional samples. Despite this limitation, the sample size aligns with those of comparable studies, especially genomic investigations. According to FAO guidelines, a minimum of 30 individuals per breed is generally recommended for livestock studies (FAO 2010). Moreover, the distinctive genomic structure of dog breeds, characterised by relatively low within-breed variability, further reduces the number of samples required to reliably represent a breed (Parker 2012). Despite this, to support the breed recognition process, a larger dataset should be subjected to detailed phenotypic evaluation to

develop a breed standard that accurately captures the existing variability within the KTKM population, thereby avoiding unnecessary restriction of the genetic pool. The collection of additional genealogical and demographic data would also be highly valuable. Accordingly, the establishment of a structured and comprehensive pedigree recording system, currently available only for a limited number of individuals, is strongly recommended, in accordance with the requirements of the FCI and national kennel clubs for official recognition and registry activation (https://www.fci.be/medias/FCI-REG-RGT-PNR-ANN-005-en-10686.pdf). Finally, systematic clinical screening should be undertaken to identify and exclude potential hereditary disorders, accompanied by the implementation of breeding programs aimed at preserving breed characteristics while preventing genetic erosion and the emergence of inherited health conditions. Altogether, these comprehensive efforts would not only ensure the scientific and administrative recognition of the KTKM breed but also contribute to the long-term preservation of a valuable genetic resource, representing both a component of canine biodiversity and a cultural heritage worthy of conservation.

Conclusions

The present study traces the first morphological and genomic description of the KTKM dog, providing scientific evidence in support of its recognition as a breed. Indeed, this population appears genomically and phenotypically homogeneous and genomically well-distinguishable from other canine breeds. Coherently with its purpose during hunting activities, it appears genetically closer to pointer-type dogs, with measurable but subordinate inputs from spaniel-type and PHAR lineages. The KTKM exhibits a distinct and uniform genomic background, reflecting its clear breed identity, while still maintaining a moderate degree of genetic variability, comparable to or greater than that of other recognised breeds. Taken together, these findings provide robust evidence supporting the official recognition of the KTKM as a distinct breed.

To ensure its long-term conservation, it will also be essential to establish genetic monitoring programs and implement carefully designed mating strategies aimed at preventing increased homozygosity and the loss of rare haplotypes, while preserving the breed's typical morphological features and functional role. These actions will promote the sustainable management and genetic health of the population, ensuring its preservation as a unique local genetic resource.

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Ethical approval

All breeders participating in this project signed a cooperation agreement. All procedures followed Italian legal regulations (National Directive n. 26/14—Directive 2010/63/UE) and the Association for the Study of Animal Behaviour (ASAB) guidelines for animal treatment in behavioural research and teaching.

Disclosure statement

The authors declare no competing interests.

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Data availability statement

Data is available on request.

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