

Research article

COAT COLOUR POLYMORPHISM AND SIGNS OF HYBRIDISATION IN GOLDEN JACKALS (*Canis aureus*) IN TÜRKİYEGizem Turan¹, Yasin Demirbaş^{1*}, Ayça Özkan Koca², Dejan Beuković³¹Department of Biology, Faculty of Engineering and Natural Sciences, Kırıkkale University, Yahşıhan, Kırıkkale, Türkiye²Department of Gastronomy and Culinary Arts, Faculty of Fine Arts, Maltepe University, Maltepe, İstanbul, Türkiye³Department of Animal Science, Faculty of Agriculture, University of Novi Sad, Serbia*Corresponding author: ydemirbas71@hotmail.com; yasdemirbas@kku.edu.tr

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ABSTRACT

In this study, the coat colour polymorphism in the golden jackal (*Canis aureus* Linnaeus, 1758) was studied on 29 specimens from 19 different localities in Türkiye. Geographically distinct coat colour variations were detected among these individuals. In addition, a jackal specimen with partially atypical morphology, which is a potential signal of jackal-wolf hybridisation, was observed in Kırklareli province in Turkish Thrace. Various biological samples of this specimen were analyzed genetically and morphologically to determine its ancestry. Our morphological evaluation, together with mitochondrial (mt) DNA nucleotide sequence BLAST results, indicated the golden jackal origin of this specimen. The coat colour polymorphism found in golden jackals in this study may indicate local adaptations specific to the locality or different phylogenetic lineages. Therefore, studies of the phylogenetic relationships of the Turkish golden jackal need to be conducted on a broad scale and incorporate mtDNA markers. Additionally, uniparental and biparental molecular markers should be used to investigate possible origins of individuals with atypical morphology, which potentially indicate genetic hybridisation of the Turkish golden jackal with other *Canis* species.

Key words:

Canis aureus, coat colourations, sign of hybridisation, mtDNA control region, Türkiye

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INTRODUCTION

The golden jackal (*Canis aureus*) has a broad distribution range in Asia Minor as well as Europe, the Middle East and Asia. It is a medium-sized mesocarnivore with an opportunistic lifestyle and is one of four naturally occurring Canidae species in Türkiye (Hoffman et al., 2018; Özkurt and Bulut, 2020; Demirtaş, 2023). Males are generally larger in body mass than females (Csányi and Sándor, 2024).

Golden jackals inhabit a wide variety of habitats, including deserts, temperate and tropical forests, grasslands, mangroves and agricultural lands. Although their diet primarily consists of small rodents, birds and their eggs, reptiles, amphibians, fish, insects and fruits, they are generally omnivorous. Jackals are skilled predators that exploit all available food sources. Their conservation status is currently listed as Least Concern on the IUCN Red List (Hoffman et al., 2018).

Golden jackals are distributed in small groups throughout almost the entire geography of Türkiye, with particularly dense populations in Turkish Thrace, Western Anatolia, the Black Sea region and the southern coasts of Anatolia (Özkurt and Bulut, 2020). Despite their recent population growth and range expansion (Ambarlı et al., 2016; Stefanović et al., 2021; Bogdanowicz et al., 2025; Srinivas et al., 2025), phenotypic data on golden jackals in Türkiye remain limited (Özkurt and Bulut, 2020). Accurate identification of Canidae species today is challenging due to factors such as morphological convergence, behavioural plasticity, limitations in traditional taxonomy and possible hybridisation events (Cányi and Sándor, 2024).

Phylogenetic and genetic information on Turkish golden jackals is still limited. However, previous studies have reported that Turkish golden jackal specimens possess relatively high genetic diversity and are clustered within two major haplogroups covering Europe and Asia (İbiş et al., 2015; Aksöyek et al., 2017; Demirtaş, 2023; Gürkan et al., 2023; Sosale et al., 2023; Milanlou et al., 2024). Rutkowski et al. (2015) reported that gene flow persists between the golden jackals of Southeast Europe and the Caucasus, with both populations contributing to the recently emerged Baltic jackal population. They also reported that the golden jackal from Samos, located near the Turkish coast, is genetically distinct or potentially related to the Caucasus population. Based on morphometric characteristics, Stoyanov (2020) concluded that golden jackals from Europe, Anatolia and the Caucasus belong to a single subspecies, *Canis aureus moreotica*. Demirtaş (2023) suggested that Turkish jackals could be a source population for both European and Caucasian lineages. Based on analyses of the final intron region of the Y chromosome Zfy, Gürkan et al. (2023) indicated that *C. aureus cruesemanni*, known as an Asiatic subspecies, is relatively different from the European jackal population. In parallel with Gürkan et al. (2023), Sosale et al. (2023) noted that the *C. aureus cruesemanni* population found in Southeast Asia is a distinct and early-diverging lineage among golden jackals and therefore should be recognised as a potential subspecies. Furthermore, the researchers reported that a golden jackal from Antalya, Türkiye (*C. aureus moreotica*), does not represent a distinct lineage due to its close genetic affiliation with individuals from India and Israel.

Milanlou et al. (2024) noted that the golden jackal rapidly dispersed across Europe with its mtDNA H1 haplotype, identifying H1 as a pioneer haplotype facilitating the expansion of the species into Europe. Their phylogenetic analyses showed that golden jackal haplotypes were monophyletic, though Indian and Eurasian haplotypes were almost unique. They also stated that the distribution and frequency of haplotypes in the golden jackal supported a rapid large-scale expansion of the species and that unique haplotypes in certain regions in Türkiye may reflect local adaptation. The phylogenetic patterns given by Milanlou et al. (2024) were consistent with those described by Demirtaş (2023).

Molecular and evolutionary studies indicated that wolves, jackals and dogs shared genomic elements, with significant gene flow among different Canid species (Moura et al., 2014; Leonardo et al., 2014; Galov, 2015; Pilot et al., 2018; Stronen, 2022; Ninausz et al., 2023; Stefanović et al., 2024; Kazimirov et al., 2024). Documented cases of golden jackal hybridisation with both dogs and wolves may impact species identification, alter population dynamics and genetic diversity, thereby posing challenges for conservation strategies (Cányi and Sándor, 2024).

The aim of this study is to investigate the coat colour variation in 29 golden jackals (*Canis aureus*) from Türkiye and to assess whether a jackal specimen from Kırklareli province in the Thrace Region – exhibiting atypical coat characteristics (possibly a jackal-wolf phenotype) – represents a hybrid individual.

MATERIAL AND METHODS

Biological sample collection and genomic DNA extraction

This study documents the coat colouration patterns of 29 golden jackal (*Canis aureus*) specimens collected from 19 distinct localities across Türkiye between 2022 and 2024 (Figure 1). The specimens included injured individuals admitted to Wildlife Protection and Rehabilitation Centres in Istanbul, Bursa, Kocaeli, Afyon, Mersin and Sinop, as well as legally hunted specimens obtained during authorised hunting periods in the provinces of Balıkesir, Tekirdağ and Kırklareli. The research was conducted with official permission granted by the General Directorate of Nature Conservation and National Parks under the Ministry of Agriculture and Forestry (Permit No: E-21264211-288.04-5395224).

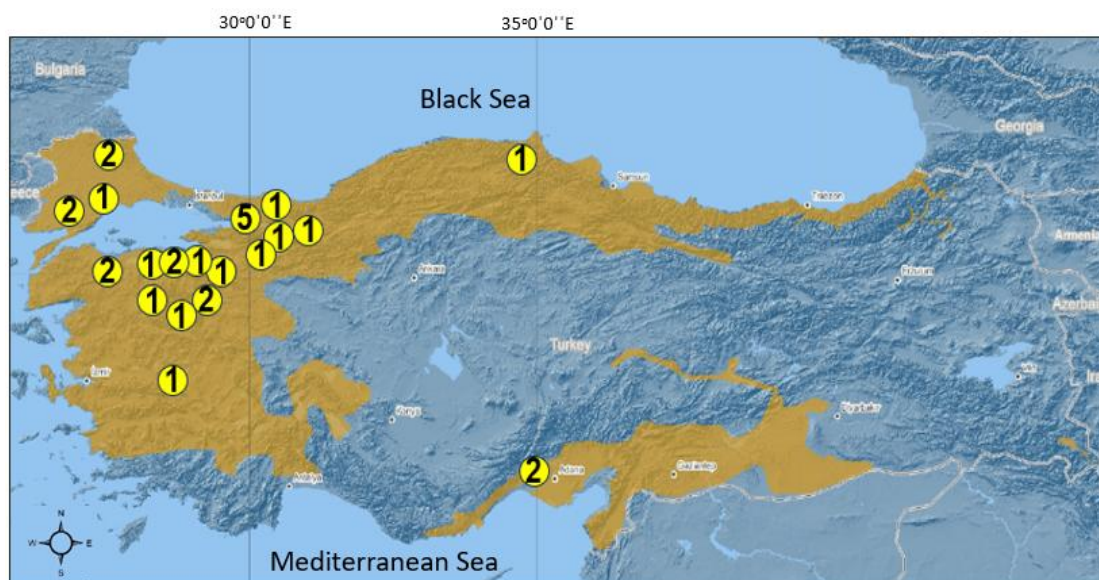


Figure 1. Localities of golden jackal samples with recorded coat colour from Türkiye. Numbers in yellow circles indicate sample numbers and brown areas show the distribution range of golden jackals in Türkiye [The map is modified from Ambarlı et al. (2016)]

In December 2023, a golden jackal individual showing a hybridisation signal with an atypical phenotype (possible wolf-jackal hybrid) was hunted in Kırklareli province. This individual was photographed, and its skull and ear tissue were collected. The skull was boiled in a 15% ammonia solution at 80°C for one and a half hours in the laboratory and cleaned with forceps. Following the protocol by Korablev et al. (2024), 17 skull measurements were taken using a caliper with a precision of 0.05 mm. In order to assess potential hybridisation signals, the skull and tooth morphology were compared in detail with *Canis aureus*, *Canis lupus* and *Canis familiaris* using the identification keys of Canidae species given in Harrison and Bates (1991), Kargopoulus (2018) and Korablev et al. (2024). Based on the degree of incisor and canine tooth wear, the individual was estimated to be 2 years old (Gipson et al., 2000). Moehlman and Hayssen (2018) reported that the skull morphologies of young and adult golden jackal individuals overlap. Coat colour characteristics of Turkish golden jackals were assessed according to Harrison and Bates (1991), Özkurt and Bulut (2020), Ninausz et al. (2023) and Barash et al. (2023).

Genomic DNA was isolated using the Gene Matrix Bacteria and Tissue Isolation Kit (EURx Ltd., Poland). DNA purity was assessed using a NanoDrop device. For optimal purity, the absorbance ratio at 260/280 nm should be within the range of 1.8–2.0. The DNA sample had a ratio of 1.98, indicating high purity. The DNA concentration was measured at 773.9 ng/μl. The genomic DNA sample was stored at -20 °C until further analysis.

Partial mitochondrial DNA sequencing for the Kırklareli sample

The mitochondrial DNA control region of the Kırklareli specimen was sequenced to investigate possible signs of hybridisation. Amplification was performed with primers described by Kirschning et al. (2007) (DLH 5'-CCTGAAGTAAGAACCAGATG-3' and LF15926F 5'-ATATAAAATACTTTGGTCTTGTAAC-3'), obtaining a 461 base pair amplicon. The PCR reaction mixture volume was 25 μl, containing 2 μl DNA diluted to the concentration 50 ng/μl, 4.5 μl of Solis Biodyne HOT FIREPol® Blend Master Mix [Ready to load with 15mM MgCl₂, 5x (Teaduspargi 9, 50411 Tartu, Estonia)], 1.25 μl of each primer diluted to 10 μM, and 16 μl PCR water (nuclease-free water). PCR was conducted in a Kyrtec SuperCycler instrument (Life Sciences, Seoul Korea). The PCR profile was the following: 95 °C for 13 min, 35 cycles of 95 °C for 45 s, 56 °C for 40 s, 72 °C for 45 s, and a final extension at 72 °C for 5 min. PCR products were checked using electrophoresis on 1.2% agarose gels and purified using ExoSAP (Fermentas) following the manufacturer's recommendations. Sequencing was conducted on an ABI 3100 Genetic Analyzer (Applied Biosystems). To determine the maternal lineage of this sample, the obtained 461 bp mitochondrial D-loop sequence was analyzed using Standard Nucleotide BLAST Tool in GenBank (<https://blast.ncbi.nlm.nih.gov>).

RESULTS AND DISCUSSION

Coat colour polymorphism

Distinct external phenotypes (i.e. coat colours) were recorded among the jackal specimens from different regions of Türkiye. In particular, individuals from the provinces of Kocaeli, Sakarya, Bursa, Tekirdağ and Sinop in the northern part of Türkiye were phenotypically similar, with a reddish brown coat colour. In contrast, the individuals from the provinces of Uşak and Mersin in the southern part of Türkiye were predominantly greyish and blackish in colour (Figure 2). From the northern to the southern regions, the dorsal part retained a black coat, while the reddish-brown colouration on the sides of the body diminished. One specimen from Kırklareli with an atypical coat colouration had a pale yellow coat on the front part of the body and a reddish brown coat on the remaining back part. The front legs of this individual were also pale yellow (Figure 2A).

Skull and dental morphology

Table 1 presents the cranial measurements of the Kırklareli golden jackal specimen characterised by atypical coat colouration. It was determined that this specimen was a golden jackal, based on the following features: the condylobasal length was 140.80 mm and the mandibular length was 110.20 mm, the nasal tips were slightly tapered forward, the cingulum on the outer side of the upper first molar (m1) was well developed and prominent, the posterior end of p4 was aligned with the anterior end of m1 and the orbital angle ranged between 45° and 48° (Figure 3).

Table 1. Skull measurements of the golden jackal from the Kırklareli province, Turkish Thrace

Number	Measurement description	Value (mm)
1	Maximum skull length	150.30
2	Condylobasal length of the skull	140.80
3	Maxillary length	120.20
4	Total length of the maxillary dentition	80.80
5	Alveolar length of maxillary molars	56.50
6	Maximum length of the P1 crown of the upper jaw	4.65
7	Maximum length of the M2 crown of the upper jaw	10.45
8	Zygomatic width	76.90
9	Width of postorbital processes	35.40
10	Length of the mandible along the articular process	110.20
11	Total length of the lower jaw dentition	77.50
12	Alveolar length of a row of molars of the lower jaw	61.50
13	Width of the head of the articular process of the mandible	16.10
14	Length from the anterior edge of the incisor alveolus to P3 upper jaw	44.00
15	Length from the anterior edge of the incisor alveolus to P4 upper jaw	44.00
16	Postorbital constriction	22.90
17	Length of the mandible along the angular process	110.10

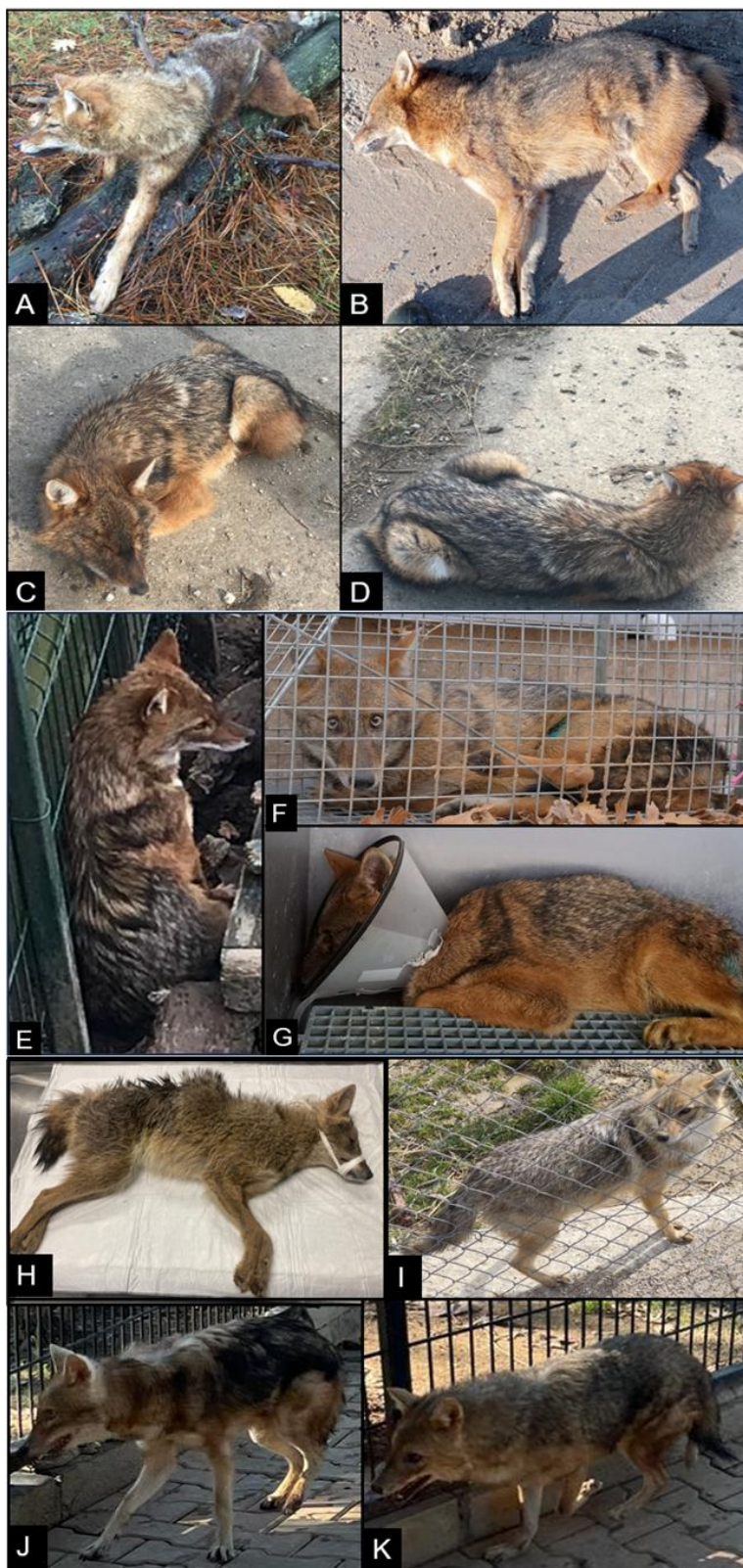


Figure 2. Some phenotypes of the golden jackal in Türkiye: A - Kırklareli specimen; B - Kırklareli-Demirköy specimen; C - Sinop specimen (lateral view); D - Sinop specimen (dorsal view); E - Tekirdağ specimen; F - Sakarya specimen; G - Bursa specimen; H - Kocaeli specimen; I - Kütahya specimen; J - Mersin specimen; K - Mersin specimen

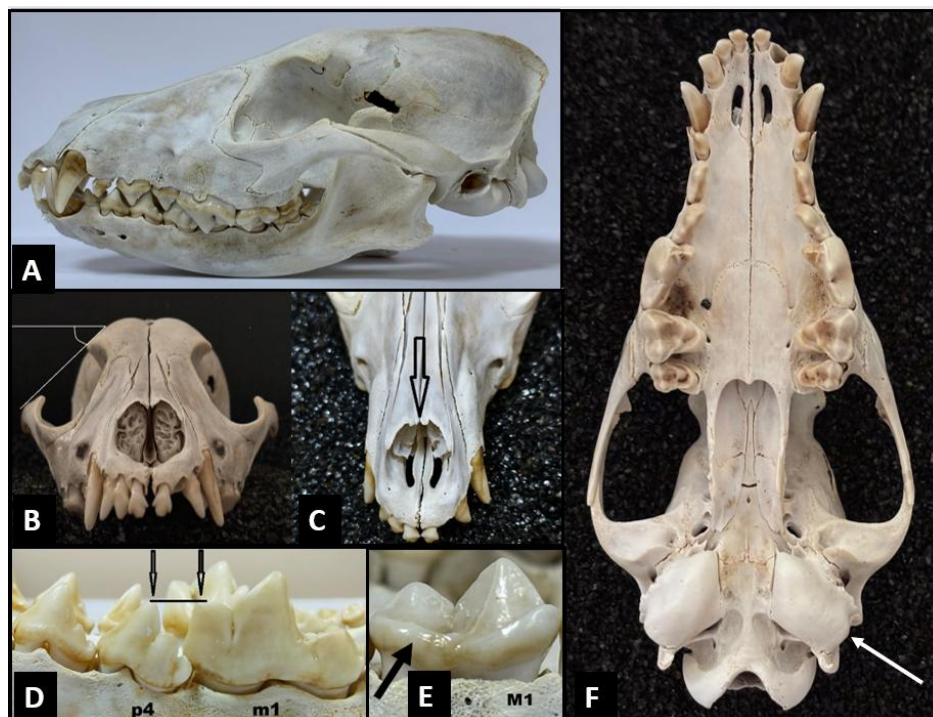


Figure 3. Taxonomic features distinguishing the golden jackal from the grey wolf and dog: A - lateral view of the skull; B - frontal view showing the orbital angle; C - terminal middle parts of the nasals; D - lateral view of the mandible showing p4 and m1; E - the outer tubercle (cingulum) of the upper first molar (m1); F - ventral view of the skull showing the auditory bullae

Partial mitochondrial DNA sequencing for the Kırklareli sample

A similarity search in GenBank resulted in 100% identity matches to golden jackal sequences previously reported from Serbia (Zachos et al., 2009), Bulgaria (Yumnam et al., 2015) and from Bartın, Tokat, Hatay, Sinop, Düzce, Ordu and Rize provinces in Anatolia (Demirtaş, 2023), indicating its maternal golden jackal origin.

Taxonomic and genetic studies on golden jackals in Türkiye are quite limited (İbiş et al., 2015; Aksöyek et al., 2017; Sosale et al. 2023; Demirtaş, 2023; Milanlou et al., 2024; Özkurt, 2024). Recent mtDNA-based phylogenetic studies have found that Turkish golden jackals are represented by a large number of haplotypes, unlike the European golden jackal, and these haplotypes come from different geographical regions in Türkiye (Demirtaş, 2023; Milanlou et al., 2024). Sosale et al. (2023) stated that Turkish golden jackal is probably not a distinct lineage due to its close genetic relationship with golden jackals from India and Israel. Demirtaş (2023) reported that the Turkish golden jackal was historically related to European and near-Asian jackals. Milanlou et al. (2024) noted that the observed golden jackal haplotype variations in Türkiye indicate local adaptations rather than a genetic lineage. The coat colour diversity detected in this study is consistent with the haplotype diversity previously found in Turkish golden jackals by Demirtaş (2023) and Milanlou et al. (2024). In particular, the relationship between different haplotypes detected in the northern and southern latitudes of Türkiye and different coat colour traits should be further investigated within comprehensive studies. In addition, the coat colours of golden jackal individuals from the northern parts of Türkiye, especially Turkish Thrace, are similar to the brown coat-coloured golden jackal individuals reported in Hungary by Ninausz et al. (2023). A detailed study on golden jackal coat colouration patterns of in Samos, Greece, found phenotypic characteristics consistent with the coat colours of golden jackal samples from the northern part of Türkiye (Custers et al., 2024).

Unravelling the origins of phenotypic variation in natural animal populations is a challenging research topic for zoologists. Phenotypic variation among canids is mainly attributed to inter-taxon hybridisation or de-novo mutations, although phenotypes resulting from human domestication also contribute to the observed diversity. In the golden jackal, atypical morphological features such as white markings, black or white colouration, merle pattern, upturned tail, long round-tipped ears and long thick coat resembling that of domesticated mammals are considered as signs of interspecific hybridisation (Ambarlı and Bilgin, 2013; Galov et al., 2015; Ninausz et al., 2023; Barash et al., 2023). Wolves are known to interbreed with other canid species, especially in areas where their ranges overlap and when

population declines limit mate availability (Hailer and Leonard, 2008). Moura et al. (2014) recorded hybridisation of wolves with dogs and jackals in Bulgaria, attributing the high frequency of hybridisation with other canid species to irregular hunting of wolves. Their findings revealed recent hybridisation between wolves and jackals. In the present study, an individual obtained from Kırklareli, which is located very close to Bulgaria, had a partially atypical morphology, resembling a potential jackal-wolf hybrid. Morphological examinations of the Kırklareli specimen revealed its golden jackal origin, while genetic examinations indicated maternal jackal ancestry. Moura et al. (2014) stated that hybridisation could be triggered by the loss of a breeding partner close to the breeding season, which is a behavioural mechanism also leading to mating between close relatives. Given the recent expansion of jackals' distribution areas, the researchers emphasised the need for further research into possible wolf-jackal hybridisation. Galov et al. (2015) reported that interspecific hybridisation is relatively frequent in nature, with numerous cases of hybridisation between wild and domestic dogs. In their study, they combined the use of biparental genetic markers (15 autosomal microsatellites and three major histocompatibility complex (MHC) loci) and uniparental genetic markers (mtDNA control region and a Y-linked Zfy intron) to assess the mixed origin of three wild dogs with abnormal phenotypic traits. A dog with an atypical phenotype observed in Trabzon-Köprübaşı in the Black Sea region of Türkiye on January 21, 2023 attracted considerable attention on Turkish social media (Figure 4). After receiving veterinary care, the animal was released back to the region by the Köprübaşı Directorate of Nature Conservation National Parks (personal communication with the Trabzon Nature Conservation National Parks Provincial Directorate). This individual can be easily identified as a potential dog-jackal hybrid, especially based on its phenotypic characteristics such as tail structure and colouration. According to Galov et al. (2015), uniparental molecular markers indicate that the direction of hybridisation favours mating of wild species females with domestic dog males, and that this pattern was observed in the majority of dog hybridisation cases. They also noted that the dog-jackal hybrid individual with a dog-like morphology, as in the Trabzon sample, had a jackal mother. Therefore, the specimen with a dog-like morphology recorded in Türkiye is likely to be the offspring of a jackal mother and a dog father. This, however, cannot be confirmed without genetic testing. The behaviour of hybrid individuals is largely influenced by the species of the mother. If the mother is a dog, the individual could be placed in a dog shelter and potentially adopted by someone living in a rural area who is experienced in training "difficult" dogs. If the mother is a jackal, placing the animal in a wildlife rehabilitation centre is more appropriate (personal communication with Prof. Dr. Malgorzata Pilot, Gdansk University).



Figure 4. A possible dog-jackal hybrid individual found in Trabzon
(The photographs belong to Demirören News Agency - DHA)

In addition, an individual with atypical morphology with black coat colour and a white area behind the ear was recorded in Mersin, Southern Anatolia. Three black patches were also observed, extending from the neck to the front

leg line (up to the front of the chest area). The chest area and the front right leg of this individual were white (Figure 2J). Although Mersin individual resembled an adult Czechoslovakian wolfdog, due to the lack of biological samples, no further examination could be conducted.

CONCLUSION

This study represents the first record of the coat colour polymorphism and atypical morphologies in golden jackals in Türkiye. Although geographical ranges of grey wolves, golden jackals and free-ranging dogs overlap significantly in Türkiye, no hybridisation studies have been conducted in areas where these species coexist. In order to determine the population genetic structure, phylogeny and possible origins of individuals with atypical (abnormal) morphology, it is necessary to conduct comprehensive genetic and morphologic analyses on a broad scale of Turkish golden jackal. Furthermore, these findings may provide valuable information for conservation management strategies for golden jackal.

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Conflict of interest: The authors declare that they have no conflict of interest.

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