Research Article

Evaluation of some morphological and genetic characteristics of Çatalburun dog breed

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Abstract: Çatalburun (Fork-nose) dogs are the members of Turkey›s indigenous gene pool. Although there are some studies on phenotypic identification of this breed, there is no study on genetic characterization. This study aimed to define the detailed morphological and genetic characteristics of this breed. For this purpose, initially, some morphological traits in 100 Çatalburun dogs were measured. Furthermore, the blood samples were taken from a total of 62 animals to identify genetic characteristics. The mean live weight was found as 20.20 kg, and the means of withers height, rump height and body length were measured as 48.2, 49.1 and 54.1 cm, respectively. In the microsatellite analysis, the mean of inbreeding coefficient (F_{IS}) was calculated as 0.048. The observed (Ho) and the expected heterozygosity (He) values were determined as 0.743 ± 0.12 and 0.744 ± 0.11, respectively. Mitochondrial DNA (mtDNA) sequence analyses revealed that *AHT 137* loci (12 alleles) and *REN247M2* loci (4 alleles) were found to have the highest and the lowest frequencies. The highest frequencies in A and B haplogroups were found to be A18 (14.52%) and B1 (59.68%) haplotypes, respectively. The factorial similarity analysis denoted that the examined dogs may be grouped in closely-related two parent lines. Although the heterozygosity values were found to be relatively high, conversely, the mutation or nucleotide diversities were found to be low. This is the first study to comprehensively describe the genomic diversity and population structure of the Çatalburun breed. Special attentions should be taken to the protection of this breed as soon as possible.

Key words: Çatalburun breed, morphological characteristics, genetic characteristics, mtDNA, pointer dog

1. Introduction

Although phenotypic studies are important in the classification of dog breeds and explanation of the kinship relations among the breeds, genetic study data can show that seemingly unrelated species may be into the same breeds. Therefore, in recent years, genetic studies are frequently preferred in classifications within breed or among the breeds [1,2].

Çatalburun (Fork-nose) breed has been raised for hunting and guarding for many years in Turkey, especially localized in Mersin province and adjacent area [3]. These animals are being raised in regions that have a Mediterranean climate, especially in low altitude areas that are very hot and humid in summer. The most distinctive feature of these dogs, which had long and drooping ears and coated with any colour combinations of brown or brown-white [4], is having a nose seem to be splitted into two parts (Figure 1). Because of this anatomic structure, it is reported that their olfactory perception has been considerably evolved to search, find, and point a prey [5]. As a pointer breed, Çatalburun dogs can perform the point behaviour by remaining motionless, with

raising paw or heading towards a prey, after the location of a prey was spotted by them [3]. Besides, these dogs can also be used as a drug detection (sniffer) or a search and rescue dog through their ability to scent under various circumstances. Because of these characteristics, they were recently being popular. Consequently, it has been suggested that the risk of unconscious crossbreeding may lead to uncontrolled gene flow and loss of genetic diversity and their unique genetic features.

Although there are some studies to morphologically define this breed, there is no data found on its genetic characteristics. This study has been performed to determine the morphological characteristics and genetic characteristics of Çatalburun dogs, which have been an important member of the indigenous gene pool of Turkey.

2. Materials and methods

2.1. Samples

This study was performed with the approval of the Ethics Committee of Ankara University (protocol number: 2013-

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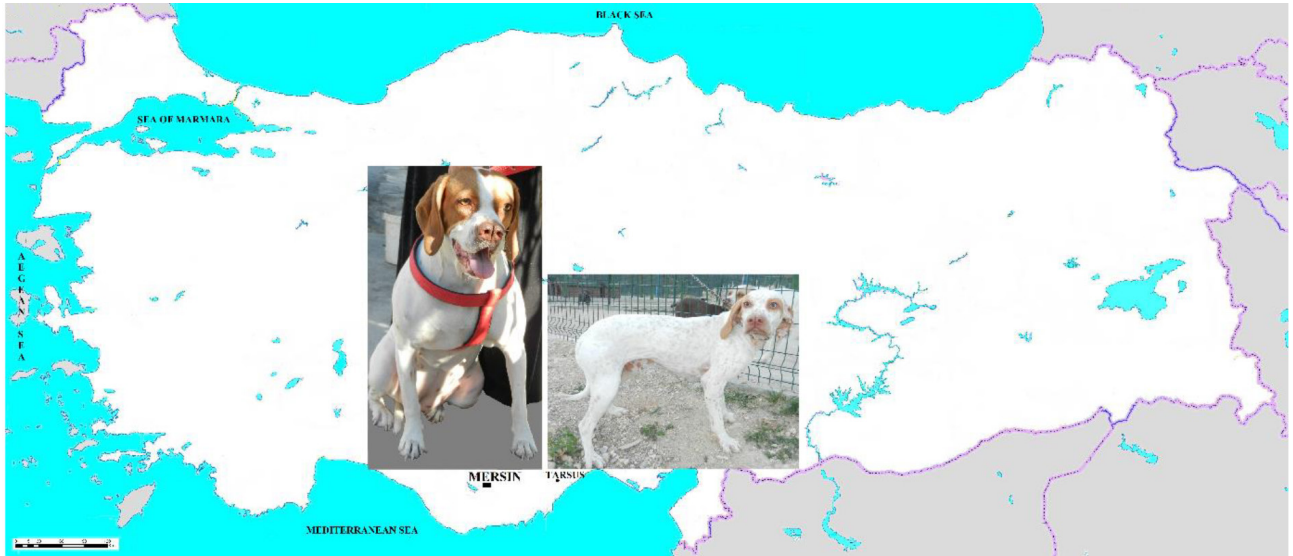
Figure 1. Çatalburun dogs and the territories where the samples are collected[1].

[1] Republic of Turkey, General Directorate of Mapping; https://www.harita.gov.tr/uploads/files/products/dilsiz-turkiye-mulki-idare-bolumleri-haritasi-1220.jpg. Aug. 22, 2021.

18-135). The morphological traits were measured in a total of one-hundred pedigreed, 12 month of age or elder Çatalburun dogs, which were raised in Mersin province (latitude, 36.812103 N; longitude, 34.641479 E) and adjacent area (Tarsus). A total of sixty-two blood samples were intentionally taken from outbred dogs by considering their pedigrees. In this manner, the blood samples of each individual are thought to be the best representatives of their breed characteristics. It was observed that all of the trial animals were individually kept in kennels or in boxes. Females in heat are held together with proper males for three days to ensure pedigreed breeding. Although the daily diets vary within the bounds of owners' possibilities, adult ones are allowed to consume home-made and meat-based meals once a day. Live weights and morphological head and body measurements have been taken by proper methods, which were previously reported by various researchers [6–8]. SPSS software (v17; SPSSInc., Chicago, IL, USA) has been used to perform statistical analyses of the data. A p-value less than 0.05 was considered statistically significant. As an inferential statistic, t-test was applied to compare sex groups for any examined trait. The one-way analysis of variance was performed to test significance of the differences between age groups, and then Duncan's multiple range test was applied for multiple comparison of age groups [9].

Blood samples taken from *Vena cephalica antebrachii* of the dogs were placed in 10 cc tubes containing anticoagulants (EDTA). The samples were stored at –20 °C for a short term until the DNA isolation process.

2.2. Genetic studies

2.2.1. DNA isolation
The DNA isolation procedures in blood samples were performed by using an isolation kit (Thermo Scientific Co., California, USA) with conforming to the manufacturer's recommendations. The absorbance readings of the DNA were performed by using a spectrophotometer at wavelengths of 260 nm (A_{260}) and 280 nm (A_{280}) where the DNA absorbs light strongly. Isolated DNA samples were stored at –20 °C until PCR (polymerase chain reaction) analyses were performed.

2.2.2. Amplification and analysis of microsatellite markers
PCR amplification and imaging procedures were performed in accordance with a previous study [10]. Thermo Scientific Canine Genotypes Panel 1.1 kits (Thermo Scientific Co., California, USA) were used to amplify microsatellite markers, which can be used to clarify possible gene flow patterns and genetic diversity in DNA samples.

The average expected heterozygosity values were estimated by the method, which is formularized by Nei [11], whereas the coefficient of inbreeding and deviations from Hardy–Weinberg equilibrium were determined by using Genetix software (v 4.05) [12].

A factorial similarity analysis (FSA) was implemented in Genetix software (v 4.05) to detect genetic distance [13].

2.2.3. Mitochondrial DNA d-loop analysis
The displacement loop (d-loop) region, considered most polymorphic, part of the longest non-coding region

(mitochondrial control region) in animals' mitochondrial DNA (mtDNA), was amplified by using PCR analysis [14]. Sequencher (v 5.4.5) software tool (Gene Codes Corp., Ann Arbor, MI, USA) was used to edit 582 base pairs (bps) length DNA sequences. DNA sequence alignments were performed by BioEdit (v 7.0.9) aligner tool [15]. Three-sample DNA sequence data (Çatalburun MRS 1-3) were submitted to the GenBank, which is part of the International Nucleotide Sequence Database Collaboration.

MEGA X (v 10.1.7) software tool was used to compute the nucleotide diversity (π) between the examined dog populations and to compute population mutation rate (Θ) and Tajima's D statistic [16,17].

3. Results

The descriptive statistics of live weight and examined morphological body measurements, which are classified by sex and ages were given in Table 1. Live weight, height at withers and height at rump were found as 20.20 ± 0.42 kg, 48.23 ± 0.34, and 49.07 ± 0.33 cm, respectively. Depth and circumference of the chest were measured as 22.27 ± 0.16 and 59.40 ± 0.39 cm, respectively. Head length and ear length were measured as 20.05 ± 0.17 and 14.87 ± 0.16 cm, respectively. By sexes, all the differences for examined traits were found to be statistically significant, except ear width and distance between ears (p < 0.05). On the other hand, among age groups, all the differences were found to be significant except for body length, chest width, ear width, distance between ears and eyes (p < 0.05).

A total of 24 polymorphic microsatellite loci were used to genetically analyse sixty-two Çatalburun dogs. Microsatellite loci analyses results revealed that *AHT 137* loci (12 alleles) and *REN247M2* loci (4 alleles) were found to have the highest and the lowest frequencies, respectively (Table 2). It was determined that there was no Hardy – Weinberg balance in the microsatellite loci used, except for *AHT 121, REN242M2*, and *REN64 E19* alleles (Table 2). Both expected and observed (H_e and H_o), the heterozygosity values and the average heterozygosity values were also presented in Table 2. The average expected and observed heterozygosity values were calculated to be 0.744 ± 0.113 and 0.743 ± 0.124, respectively.

For each locus, a F_{IS} was calculated to determine population differentiations (Table 2). The F_{IS} value, which is the coefficient of inbreeding and denotes the average heterozygote deficiency for each population was calculated to be 4.80%.

The results of the FSA performed by Genetix software tool, which graphically projects individuals on the factor space defined by the similarity of their allelic states and used to detect the degree of similarity between possible groups, were given in Figure 2. The graphical FSA results revealed that the presence of only two groups can be mentioned in this studied population. However, only two dogs were out of these groups. Furthermore, there was no relationship between the groups and the territories (Mersin and Tarsus) where the samples were collected.

Moreover, as another approach in this study, by a length of 582 bps DNA sequences, the mt-DNA d-loop region was used to compare the genetic relationship between examined dogs. According to the mt-DNA d-loop haplotypes of the samples, it a total of six haplotypes in haplogroup A, and only one haplotype in haplogroup B were detected. Once more, in light of the mt-DNA D-loop region analysis, the frequency distributions of A11, A18, A020, A28, A80, B1, C3 and a new (undefined) haplotypes were calculated as 4 (6.45%), 9 (14.52%), 2 (3.23%), 1 (1.61%), 1 (1.62%), 37 (59.68%), 5 (8.06%) and 3 (4.84%), respectively. In conclusion, the highest frequencies of A18 (14.52%) and B1 (%59.68) haplotypes were found in A and B haplogroups, respectively.

For the nucleotide sequences of the examined population, a total of 11 different polymorphic sites were determined in the mtDNA control region. Moreover, while the polymorphism rate was 3.2%, the Tajima's D value was found to be 1.943.

4. Discussion

As above mentioned, firstly some morphological traits of the Çatalburun breed were determined in this study. Our morphological measurements, as a whole, were found to be a bit lower than the one in the report of Oğrak et al. [18], except for the height at rump, chest depth and chest width. On the other hand, in a study by Yılmaz [5] on same breed, lower body measurements than our findings were reported. In another study, which was conducted with the same dog breed, Kirmizibayrak and Takici [19] reported lower (head length, height at withers, chest girth) or higher (chest depth, distance between ears and eyes, ear length) body measurements on certain body parts compared to our study. As is well known, many factors, such as sample size, selective owner/breeder wishes and/or priorities to breeding, managerial and/or nutritional conditions can cause such phenotypic variations.

In consideration of the scientific literature review, although some morphological studies are published, for Çatalburun breed, there is no study to genetically identify them. To the best of our knowledge, this study will be the precursory one, at this point of view.

Inbreeding, in a population, is the production of offspring by mating individuals that are closely related. Inbreeding may cause a change in genotype frequencies by increasing homozygosity and reducing heterozygosity, and, thus, the Hardy–Weinberg balance will be broken. Wright's F-statistics is the most commonly used method of summarizing structure within genetic variability as

Table 1. The means and the standard errors for live weight (kg) and some morphological measurements (cm) of Çatalburun dogs.

	n	Wither height	Rump height	Body length	Chest width	Chest depth	Chest girth	Front cannon circumference	Back cannon circumference	
Age (mo)		**	*	-	-	**	***	***	***	
12	28	47.09 ± 0.69[a]	48.06 ± 0.67[a]	52.90 ± 0.65[a]	18.89 ± 0.41	21.19 ± 0.32[a]	56.59 ± 0.77[a]	8.70 ± 0.13[a]	8.21 ± 0.11[a]	
13–24	23	48.37 ± 0.65[b]	49.48 ± 0.64[b]	54.28 ± 0.63[ab]	19.27 ± 0.39	22.94 ± 0.30[b]	60.11 ± 0.74[b]	9.39 ± 0.12[b]	8.55 ± 0.11[b]	
25–36	17	48.68 ± 0.84[b]	49.44 ± 0.82[b]	54.23 ± 0.79[ab]	19.66 ± 0.49	22.55 ± 0.39[b]	60.44 ± 0.94[b]	9.37 ± 0.16[b]	8.62 ± 0.14[b]	
37≤	32	48.76 ± 0.56[b]	49.32 ± 0.55[b]	54.78 ± 0.53[b]	19.69 ± 0.33	22.40 ± 0.26[b]	60.45 ± 0.63[b]	9.49 ± 0.10[b]	8.78 ± 0.01[b]	
Sex		***	***	***	**	**	***	***	***	
Female	62	46.87 ± 0.41	47.92 ± 0.4	52.85 ± 0.39	18.93 ± 0.24	21.94 ± 0.19	58.57 ± 0.46	8.99 ± 0.08	8.38 ± 0.07	
Male	38	49.58 ± 0.55	50.23 ± 0.546	55.24 ± 0.53	19.83 ± 0.33	22.60 ± 0.26	60.22 ± 0.63	9.48 ± 0.11	8.71 ± 0.3	
Overall	100	48.23 ± 0.34	49.07 ± 0.33	54.05 ± 0.33	19.38 ± 0.20	22.27 ± 0.16	59.40 ± 0.39	9.24 ± 0.07	8.54 ± 0.06	
	n	Live weight	Head length	Face length	Ear length	Ear width	Distance between ears	Distance between eyes	Mouth circumference	Witness length at point of tail
Age (mo)		**	***	**	***	-	-	-	***	***
12	28	18.62 ± 0.83[a]	19.08 ± 0.33[a]	6.92 ± 0.16[a]	13.85 ± 0.32[a]	11.36 ± 0.18	13.65 ± 0.23	4.79 ± 0.07	18.33 ± 0.26[a]	18.33 ± 0.26[a]
13–24	23	20.32 ± 0.79[b]	20.00 ± 0.32[b]	7.22 ± 0.15[ab]	15.47 ± 0.31[b]	11.70 ± 0.17	13.58 ± 0.22	4.94 ± 0.07	19.43 ± 0.24[b]	19.43 ± 0.24[b]
25–36	17	20.90 ± 1.01[b]	20.71 ± 0.41[b]	7.59 ± 0.20[b]	15.15 ± 0.40[b]	11.71 ± 0.22	13.87 ± 0.28	5.12 ± 0.09	19.80 ± 0.31[b]	19.80 ± 0.31[b]
37≤	32	20.97 ± 0.67[b]	20.41 ± 0.27[b]	7.58 ± 0.13[b]	15.02 ± 0.26[b]	11.93 ± 0.14	13.63 ± 0.19	4.87 ± 0.06	19.13 ± 0.21[b]	19.13 ± 0.21[b]
Sex		**	***	***	***	-	-	*	***	***
Female	62	19.43 ± 0.49	19.58 ± 0.20	7.07 ± 0.09	14.40 ± 0.19	11.62 ± 0.11	13.68 ± 0.14	4.84 ± 0.05	18.59 ± 0.15	18.59 ± 0.15
Male	38	20.97 ± 0.67	20.52 ± 0.27	7.58 ± 0.13	15.35 ± 0.26	11.74 ± 0.15	13.69 ± 0.19	5.00 ± 0.06	19.75 ± 0.21	19.75 ± 0.21
Overall	100	20.20 ± 0.42	20.05 ± 0.17	7.33 ± 0.08	14.87 ± 0.16	11.68 ± 0.09	13.68 ± 0.12	4.93 ± 0.04	19.17 ± 0.13	19.17 ± 0.13

-: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; [a, b, c]: Means within columns with different superscripts differ significantly ($p < 0.05$).

Table 2. Expected heterozygosity and observed heterozygosity indexes, Hardy–Weinberg equilibrium, and F_{IS} values in Çatalburun dog populations.

Locus	Heterozygosity			Hardy–Weinberg equilibrium		F_{IS}
	Allele numbers	He	Ho	P	Significance	
AHT121	11	0.820	0.900	0.000	***	−0.093
AHT137	12	0.855	0.883	0.926	NS	−0.027
AHTh13	10	0.839	0.850	0.830	NS	0.009
AHTh171	9	0.807	0.783	0.945	NS	−0.016
AHTh260	10	0.760	0.783	0.932	NS	−0.051
AHTk211	7	0.758	0.717	0.690	NS	0.009
AHTk253	7	0.728	0.783	0.534	NS	−0.075
CXX279	8	0.782	0.800	0.592	NS	−0.048
FH2001	8	0.714	0.783	0.051	NS	0.024
FH2054	8	0.774	0.683	0.213	NS	0.116
FH2328	9	0.805	0.850	0.587	NS	0.029
FH2848	9	0.804	0.754	0.869	NS	0.049
INRA21	7	0.743	0.700	0.120	NS	0.010
INU005	7	0.729	0.650	0.250	NS	0.113
INU030	5	0.687	0.623	0.995	NS	0.024
INU055	9	0.757	0.738	0.455	NS	0.004
LEI004	6	0.671	0.689	0.624	NS	−0.076
REN105L0	8	0.741	0.754	0.103	NS	0.038
REN162C0	6	0.653	0.700	0.321	NS	−0.069
REN169D0	10	0.797	0.787	0.498	NS	0.030
REN169O1	8	0.774	0.771	0.185	NS	0.027
REN247M2	4	0.276	0.279	0.013	*	0.083
REN54P11	8	0.839	0.883	0.663	NS	0.023
REN64E19	7	0.737	0.683	0.002	**	0.119
$\bar{h}_S \pm S_e$		0.744 ± 0.113	0.743 ± 0.124			0.048 ± 0.071

NS (Non-significant); p > 0.05; *: p < 0.05; **: p < 0.01; ***: p < 0.001; F_{IS}: the mean of inbreeding coefficient; He: expected heterozygosity; Ho: observed heterozygosity; $\bar{h}_S$: mean heterozygosity; Se: standard error.

measured by levels of heterozygosity into components of intra- and inter-populations variation e.g., it is used to determine inbreeding degree in subpopulations, or to estimate the selection model associated with polymorphic alleles [12]. It is obvious from Wright's formula of the F-statistic model that the parameter F_{IS} is free to take either positive or negative values depending on whether there is a deficit or excess of heterozygous genotypes (compared to the expected heterozygosity), respectively. In a previous study, which is conducted with a total of six different pointer dog breeds, such as Cesky Fousek, Deutsch Drahthaar, German Wirehaired Pointer, Bohemian Wirehaired Pointing Griffon (individual), Wirehaired Pointing Griffon, German Shorthaired Pointer, the expected and the observed heterozygosity values (0.639 to 0.683, and 0.639 to 0.669, respectively) were reported to be lower than that we currently revealed in this study (0.744 and 0.743, respectively). The value of F_{IS} (0.048) in Çatalburun breed was found to be higher than in Cesky Fousek (0.005) and in German Shorthaired Pointer (0.004) breeds [20]. For Çatalburun breed, this can be interpreted as an existence of low level inbreeding, in spite of sampling from an underpopulated local breed in terms of heterozygosity and F_{IS}, which are used to define the genetic diversity. On

the other hand, considering diversity of hunting abilities, it can be inferred that the breeders/owners are conscious of the genetic preservation of this breed.

The expected and the observed heterozygosity values were found to be higher than in Zerdava breed (0.732 and 0.712), which is raised for sport hunting [14], and also than in Turkish hound breed (0.705 and 0.710), which have been determined by using different alleles [21]. After the comparison of the current expected and observed heterozygosity values with the other study reports, which were performed by using different alleles for herding and guarding dog breeds, it was found to be higher (0.789 and 0.764) [21] or lower than Akbas breed (0.620 and 0.710), lower than Kangal breed (0.763 and 0.766), lower than Kars black and grey shepherd dogs (0.781 and 0.778; 0.794 and 0.735), and higher than Kars white shepherd dogs (0.731 and 0.664) [10].

The mtDNA analysis method helps to elucidate the phylogenetic neighbour-joining tree of relationships among populations and individuals in dogs. It is known that all of the existing dog breeds of the world are originated from six different common ancestors (haplogroups) (A, B, C, D, E and F), depending on the region where they were bred. According to the various studies, although their frequency varies (55%–100% for A; 8%–18% for B; 3%–17% for C), in today's dog breeds, the traces of A, B and C ancestors can be encountered in a wide range of intercontinental geographic regions, such as Africa, the north polar region of America, Europe, east Asia, Southwest Asia, Siberia and India [22, 23]. Nevertheless, Savolainen et al. [23] reported that B and C haplogroups were not found in America continent's existence dog breeds. They also reported that D, E and F haplogroups were mostly observed in Turkey, Spain,
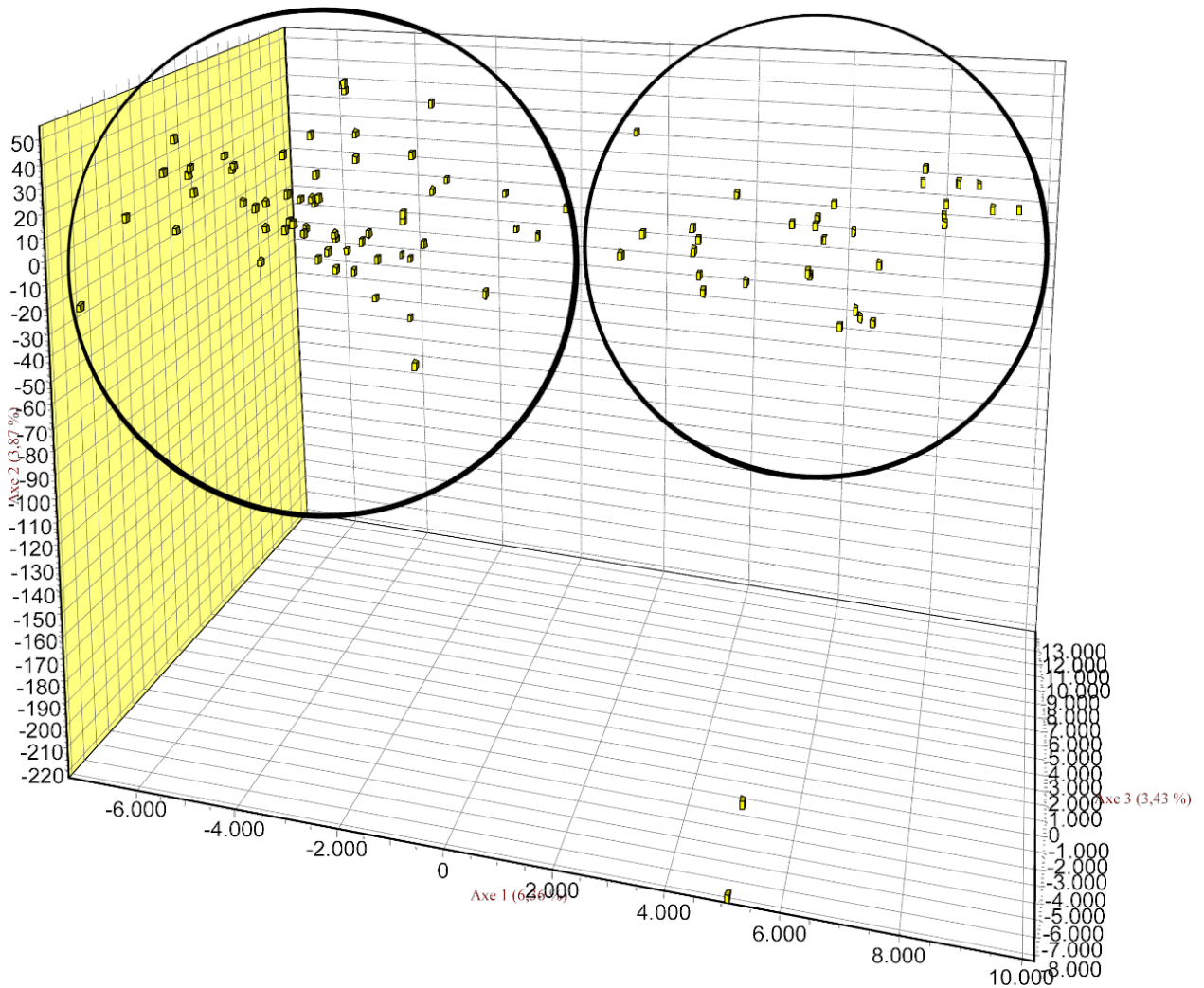
Figure 2. Factorial similarity analysis (the circles represent closely related animals according to genetic similarity).

Scandinavia, Korea, Japan and Siberia's existence dog breeds. It was reported that, while the haplogroup D is found in Southwest Asia with a frequency of 2%–10%, haplogroup E and F are found in East Asia with a frequency of 3%, and 0.5%–4%, respectively [24, 25]. In this study, A, B and C haplogroups were found in Çatalburun breed, too. These findings were found to be similar with the ones in the report of Parra et al. [26], in five different pointer dog breeds. These results reveal that the Çatalburun breed has similar/common genetic haplogroups with old world canine populations and it may be associated with other pointer dog breeds.

Considering the frequency of polymorphic site, nucleotide diversity and Tajima's D values in the mtDNA control region, low mutation or nucleotide diversity in the sampling group can denote that this breed is currently controlled bred. However, when the FSA graph was dissected (Figure 2), except two dogs, the examined dogs were genetically grouped in two close-related maternal lines. This difference can be a result of two different site consideration in both methods (mtDNA control region in Tajima's D vs. microsatellite region in FSA) and a result of two different evaluation approaches (DNA sequence vs. microsatellite length). Nevertheless, in order to elucidate this difference, further detailed genetic studies are needed to compare the results obtained with more samples and especially with morphologically similar breeds.

Considering the morphological and behavioural traits of Çatalburun breed, this breed may be classified in pointer dog breeds section according to the definitions of the Federation of Cynologique Internationale [27]. However, more comprehensive studies are needed to be performed, and it is planned to conduct new researches in this direction.

In conclusion, the morphological and genetic characteristics of breed, which is an important indigenous gene pool of Turkey, were determined. To the best of our knowledge, this is the first study to comprehensively describe the genomic diversity and population structure of this breed by exploring the distribution of inbreeding coefficient, heterozygosity and factorial similarity analysis based on microsatellite and mtDNA sequence analyses. Special attention should be taken to the protection of Çatalburun breed from now on.

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