www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article
DOI: 10.46309/biodicon.2025.1456557
18/1 (2025) 58-63

Analysis of cytochrome *c* oxidase subunit I gene of *Canis lupus* in Türkiye

Elif YILDIZ AY [*1], İrfan ALBAYRAK [1]
ORCID: 0000-0003-4549-7789**;** 0000-0000-0000-0000

[1] University of Kırıkkale, Faculty of Science and Arts, Department of Biology, 71450, Yahşihan, Kırıkkale, Türkiye

Abstract

This study is based on the analysis of the cytochrome c oxidase subunit I gene of 20 grey wolf samples obtained from different regions of Türkiye between 2014-2018. The results from this study were compared with sequences from *Canis lupus* records from other localities available in GenBank. This comparison showed that *Canis lupus* in Türkiye originates from the same genetic source as the *Canis lupus* sequences in GenBank with a very high degree of similarity in sequencing (97-100%).

Keywords: Mitochondrial DNA, cytochrome *c* oxidase, gray wolf, Türkiye

---------- * ----------

Türkiye'deki *Canis lupus*'un sitokrom oksidaz subunit I gen analizleri

Özet

Bu çalışma, 2014-2018 yılları arasında Türkiye'nin farklı bölgelerinden elde edilen 20 adet gri kurt örneğinin sitokrom c oksidaz subunit I geninin analizine dayanmaktadır. Bu çalışmadan elde edilen sonuçlar, GenBank'ta bulunan diğer bölgelerdeki *Canis lupus* kayıtlarından elde edilen dizilerle karşılaştırıldı. Bu karşılaştırmada, Türkiye'deki *Canis lupus*'un Gen Bankası'ndaki *Canis lupus* dizileriyle aynı genetik kaynaktan geldiği ve dizilim açısından çok yüksek derecede benzerlik gösterdiği (%97-100) kaydedildi.

Anahtar kelimeler: Mitokondriyal DNA, sitokrom c oksidaz, gri kurt, Türkiye

1-Introduction

With rapid developments in the field of molecular biology in the last fifty years, interspecific and intraspecific genetic relationships are increasingly examined. Mitochondrial DNA is frequently used to examine these relationships due to the reliability of gene sequencing [2]. Because mtDNA is inherited only maternally and there is no recombination of mtDNA, this has led to its intensive use in population genetics, phylogenetics, and studies of intraspecific and interspecific genetic similarity and difference [1].

Mitochondrial genome size in animals is between 16,300-17,000 bp, and the genes in the mitochondrial genome and the sequence of these genes have been very well preserved during evolution [12]. The main reason for choosing the COI gene as the standard barcode gene was its significant discrimination power for more than one species [3]. The first to apply this DNA barcode, stated that the Cytochrome c Oxidase Subunit I (COI) gene can distinguish different species at the molecular level and that this gene is the appropriate DNA barcode for the Animal Kingdom [4].

In a global study examining the phylogeography and population history of gray wolves based on mitochondrial control region sequences, two Turkish gray wolves of unknown origin were included. The analysis revealed that both samples shared a single haplotype, which is identified as the most widely distributed haplotype in the dataset. This haplotype is common across multiple regions, including Portugal, Croatia, Greece, Sweden, and Western Russia.

[*] *Corresponding author* : Tel.: +905354589402; Fax.: +905354589402; E-mail: elifyildiz_ka@hotmail.com
 Received: 25.03.2024; Published: 15.04.2025 BioDiCon. 1150-150324

Additionally, the Turkish samples were found to cluster within the same phylogenetic group as wolves from Asia, Europe, and the Middle East [11].

In a study aimed at assessing the genetic diversity and elucidating the phylogenetic relationships of gray wolves in Türkiye, 12 wolf samples from central and eastern Anatolia were analyzed. The study identified seven D-loop haplotypes (332 bp), and comparison of these sequences with those recorded in GenBank from Eurasian wolves revealed that two haplotypes were unique to Turkish wolves. The findings also indicated a high level of genetic variation within the wolf populations in Türkiye [5].

Studies based on standard molecular markers, conducted on small samples of gray wolf populations in Türkiye, have reported high genetic variation within these populations. However, it has also been noted that the available genetic data on the species remain limited and insufficient for accurately assessing and monitoring the current and future status of gray wolves in Türkiye [8].

This study aimed to determine the Cytochrome c Oxidase Subunit I gene sequence of grey wolf samples obtained from Türkiye and to compare them with data from the same species in GenBank.

2-Material and Method

2.1. Ethical Scope

For this study a research permit was obtained from the TR Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks (dated 05.04.2016 and numbered 72784983-488.04-75301). In addition, a 5-year cooperation protocol was signed between the same General Directorate and Kırıkkale University Faculty of Arts and Sciences on 15th September 2015.

2.2. Material

Tissues of 20 grey wolves (*C. lupus*) and one dog (*C.familiaris*) were used in the study. Scientific and ethical rules regarding animal rights were complied with in the sampling. All of the samples were taken from dead animals. Sampling from ear cartilage tissues material was placed in Eppendorf tubes, and numbered and stored in a deep freezer at -20˚C until use.

2.3. Oligonucleotide primers

In this study the primer sets shown in the table below were synthesised for the detection of the Cytochrome c Oxidase Subunit I gene [7] (Table 1). Primers were diluted at 100 pm/µl as the storage dilution and 20 pm/µl as the working dilution and stored at -20˚C.

Table 1. Primers and DNA sequences used in the molecular analyses [7]

Primer	DNA Sequence
U_{COI}	(5' - CAC TGC CTT GAG CCT CCT CAT C -3')
D_{COI}	(5' - GGG GAG GTT GCG TCC TGT AAT C -3')

2.4. Nucleic acid isolation

Nucleic Acid isolation was performed using a commercial kit following manufacturer's instructions (Tissue SV Mini, Cat #104-101, Lot #11717B03002, GeneAll Biotech, South Korea). The obtained nucleic acid concentration was measured with a spectrophotometer (Qubit Assay, Thermo Fisher Scientific, USA), and adjusted to 100 pg/µl using molecular-grade water. The obtained DNA was stored at -80°C until sequenced.

2.5. Polymerase Chain Reaction (PCR)

A commercial kit was used for PCR amplification (AmpMaster Kit, GeneAll, Cat no.544-010, Lot no. TM016G19001 GeneAll Biotech, Korea) following the method of Li et al. (2011). The reactions were carried out in a total volume of 20 µl (Table 2).

The results were evaluated on a UV transilluminator (Blook Led Transilluminator BK001) and photographs were taken. expected As an indication of positive PCR amplification, specific bands were expected to form in the approximately 1500 bp regions for the UCOI and DCOI primer pair. The results were recorded by in photographs.

Table 2. Composition of the reagent used in PCR

Reagent	Amount Used (µL)
MasterMix	10
Primer U_{COI} (10 pmol)	1
Primer D_{COI} (10 pmol)	1
dH_2O	4
DNA	4
Total Reaction Volume	20

2.6. DNA sequencing

DNA sequence analysis was done commercially. Positive PCR samples were ordered for duplex DNA sequence analysis. The primer sets used for PCR were used in all DNA sequence analyses. DNA sequence analysis results were received without processing.

2.7. Phylogenetic analyses

The obtained DNA sequences were analysed using BioEdit software (http://www.mbio.ncsu.edu/BioEdit/page2.html) and matching sequences were performed with ClustalX1.83 software [10]. The Blast programme was used to compare the obtained DNA sequences (http://blast.ncbi.nlm.nih.gov/Blast).
In this study it was aimed to determine genetic similarities of the mitochondrial COI gene in the sampled Turkish wolf population compared with other grey wolves. The obtained DNA sequences were recorded in the GenBank database. Sequences previously obtained by other researchers (KU644671.1, KF661055, KU696410, KU696398) were also used to compare these DNA sequences. Dendrograms of these DNA sequences were prepared according to the "neighbor-joining" method.

3- Results

For this study molecular analysis of a total of 21 samples was performed and COI gene sequences of different lengths (1362-1416) were obtained. Appropriately sized gene regions were obtained in a total of 16 samples. PCR was unsuccessful in four samples (4, 11, 12, 22) and one sample did not have a sufficiently long sequence reads (26), so that sequences were unavailable for five of the samples.

Directories matched with the Bio-Edit program were checked with Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi). As a result of matching with the BioEdit program, a partial sequence of the COI gene with a length of 1400 bp was obtained for each useable sample (Table 4). The COI gene sequences obtained in this way were submitted to the “GenBank” (https://www.ncbi.nlm.nih.gov/genbank/) and the following Accession Numbers were obtained (Table 3)As a control for this gene, DNA sequences of wolf samples from different localities (Croatia, Iran, Israel, Canada) were obtained from GenBank and comparisons were made [6], [9].

Table 3. List of grey wolf specimens examined

Sample No	Locality	Accession Number	Reference
Wolf 2	Eskişehir	MK461173	This study
Wolf 3	Çankırı	MK461174	This study
Wolf 6	Bitlis	MK461175	This study
Wolf 7	Çankırı	MK461176	This study
Wolf 8	Bayburt	MK461163	This study
Wolf 9	Bayburt	MK461164	This study
Wolf 10	Bayburt	MK461162	This study
Wolf 11	Bayburt	MK461177	This study
Wolf 16	Çankırı	MK461165	This study
Wolf 17	Ankara	MK461166	This study
Wolf 19	Çankırı	MK461168	This study
Wolf 21	Ankara	MK461167	This study
Wolf 24	Çorum	MK461169	This study

Table 3. Continued

Wolf 25	Çankırı	MK461170	This study
Wolf 27	Çankırı	MK461171	This study
Wolf 28[1]	Çankırı	MK461172	This study
Iran2	Iran	KU644671.1	[6]
Israel2	Israel	KF661055	[9]
WCRO7	Croatia	KU696398	[6]
Can11	Canada	KU696410	[6]

[1] This specimen was identified as a dog according to the results of the DNA sequence analysis.

Table 4. DNA quantification results

Sample No	Concentration
2	22.4 µg/ml
8	11.2 µg/ml
17	6.03 µg/ml
4	0,73 µg/ml
11	1.84 µg/ml
12	0.35 µg/ml
22	0.19 µg/ml

The relationships between these samples are shown in a phylogenetic tree, both without outgroup determination (Figure 1) and with KU644671.1 [6] as an outgroup (Figure 2).

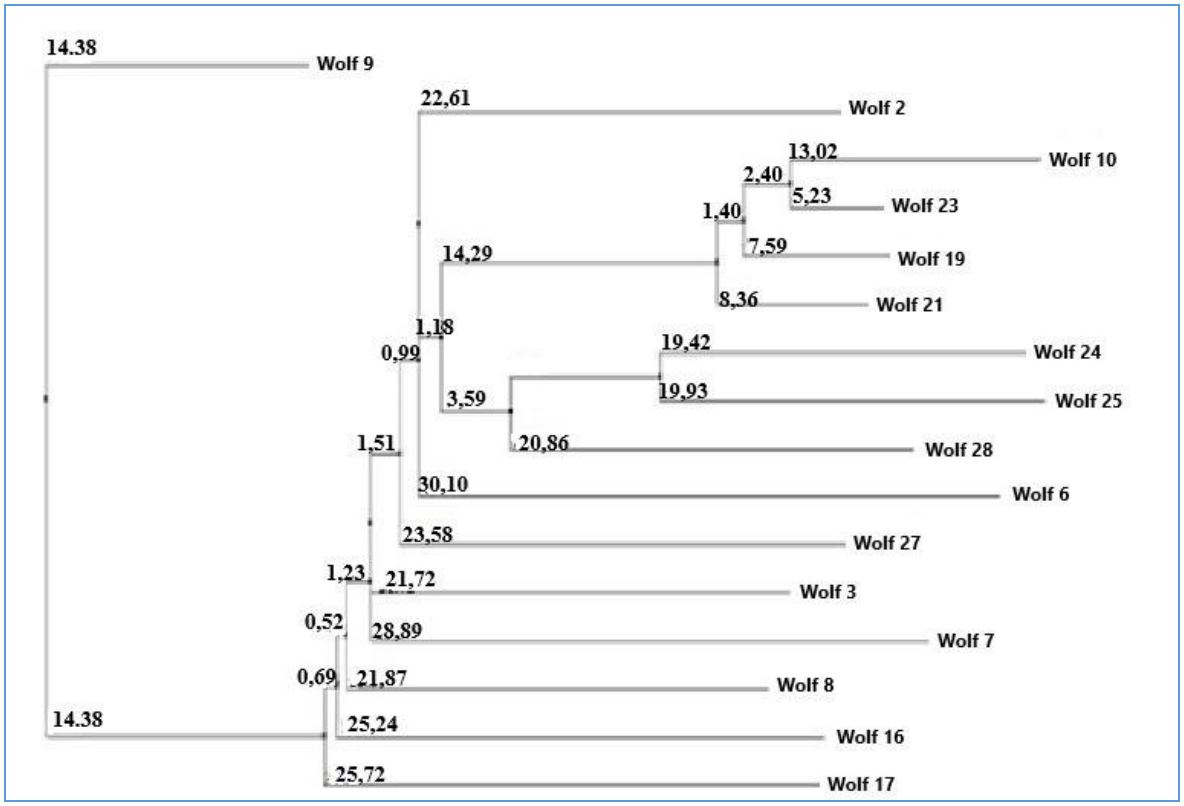

Figure 1. Phylogenetic analysis of samples (without outgroup determination)

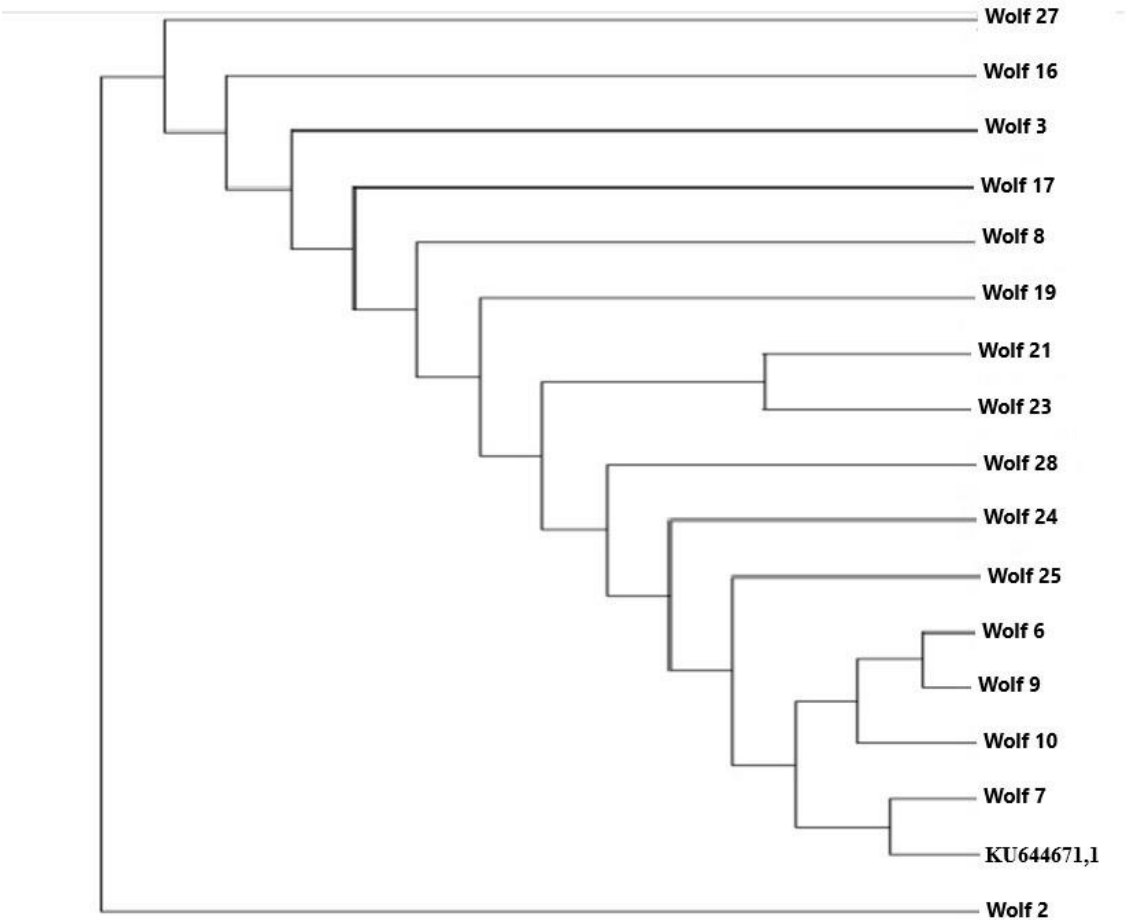

Figure 2. Phylogenetic analysis of the samples (by determining the outgroup)

4- Conclusions and Discussion

In the statistical analysis, the obtained DNA sequences are very similar to the wolf sequences registered in GenBank (97-100%). Again, there is sufficient data to be able to distinguish genetically from dogs. This study is unique in terms of determining the sequence the mitochondrial gene region (COI) and carry out an intraspecific phylogenetic analysis of a wild species in Türkiye.

Genetic information on the gray wolf population in Türkiye has been found to be highly limited and insufficient for effectively monitoring the current and future status of the species [8]. In this context, the contribution of additional genetic data from Türkiye, particularly by uploading the results of this study to the GenBank database, is of significant value. For future research, whole-genome sequencing of Turkish wolf samples is essential to enable more detailed evaluations and comparisons, enhancing our understanding of their genetic diversity and evolutionary history.

Acknowledgements

We thank Prof. Dr. Hikmet ÜN and Dr. Andrew C KITCHENER for his contribution to our work.

References

[1] Avise, J.C. (2004). *Molecular Markers, Natural History, and Evolution*. *2nd Ed.* Sunderland, MA; Oxford: Oxford University Press. http://dx.doi.org/10.1007/978-1-4615-2381-9

[2] Fonseca, R.R., Johnson, W.E., O'Brien, S.J., Ramos, M.J., Antunes, A. (2008). The adaptive evolution of the mammalian mitochondrial genome. BMC Genomics. 9 (119):1-22. https://doi.org/ 10.1186/1471-2164-9-119

[3] Hebert, P.D.N., Ratnasingham, S., Dewaard, J.R., 2003a. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings Of The Royal Society B: Biological Sciences. 270:96-99.

[4] Hebert, P.D.N., Cywinska A, Ball SL, deWaard JR (2003b). Biological identifications through DNA barcodes. Proceedings Of The Royal Society of London Series B: Biological Sciences. 270: 313-321. https://doi.org/ 10.1098/rspb.2002.2218

[5] İbiş, O., Aksöyek, E., Özcan, S., Keten, A., Yorulmaz, T., & Tez, C. (2016). Genetic analysis of the Turkish gray wolf (Canis lupus) based on partial mitochondrial DNA sequences. Vertabrate Zoology, 66 (3), 427-435.

[6] Koblmuller, S., Vila, C., Lorente-Galdos, B., Dabad, M., Ramirez, O. et al. (2016). Whole mitochondrial genomes illuminate ancient intercontinental dispersals of grey wolves (*Canis lupus*). Journal of Biogeography, 43 (9): 1728-1738. https://doi.org/ 10.1111/jbi.12765

[7] Li, Y., Zhao, X., Pan, Z., Xie, Z., Liu, H. et al. (2011). The origin of the Tibetan Mastiff and species identification of Canis based on mitochondrial cytochrome c oxidase subunit I (COI) gene and COI barcoding. Animal, 5 (12): 1868-1873. https://doi.org/ 10.1017/S1751731111001042

[8] Ölmez, B., & Demirbaş, Y. (2024). Türkiye'deki Canis lupus (Gri Kurt)'un Dağılımı ve Genetik Yapısı. International Journal of Engineering Research & Development (IJERAD), 16(2). https://doi.org/10.29137/umagd.1416917

[9] Thalmann, O., Shapiro, B., Cuıv, P., Schuenemann, V.J., Sawyer, S.K. (2013). Complete Mitochondrial Genomes of Ancient Canids Suggest a European Origin of Domestic Dogs. Science, 342 (6160): 871-874. https://doi.org/10.1126/science.1243650

[10] Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G. (1998). The CLUSTAL_X Windows Interface: Flexible Strategies for Multiple Sequence Alignment Aided by Quality Analysis Tools. Nucleic Acids Research, 25 (24): 4876-4882. https://doi.org/10.1093/nar/25.24.4876

[11] Vilà, C., Amorim, I. R., Leonard, J. A., Posada, D., Castroviejo, J., Petrucci-Fonseca, F., ... & Wayne, R. K. (1999). Mitochondrial DNA phylogeography and population history of the grey wolf Canis lupus. Molecular Ecology, 8 (12), 2089-2103. https://doi: 10.1046/j.1365-294x.1999.00825.x

[12] Zahn, L.M. (2016). Effects of mtDNA and nuclear genetic variation. Science, 353 (6305): 1246-1247. https://doi.org/ 10.1126/science.353.6305.1246-c.