Research Article

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Phylogenetic Analysis of Some Wild Birds in Şanlıurfa (Türkiye)

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ABSTRACT

The increase in population worldwide causes an increase in anthropogenic effects such as agriculture and industry, and as a result, the habitat of many naturally living species is changing. Because of this change, species that adapt to the environment survive and those that cannot adapt to the environment either had to migrate in order to survive, or their numbers decreased and their generations were endangered. In Şanlıurfa, both the construction of dams and the increase in agricultural activities significantly affect bird diversity. Therefore, it is important to take precautions by investigating their phylogenetic origins and migration routes. The aim of the present study is to perform phylogenetic analysis using mtDNA COI marker for some wild bird species in Şanlıurfa. Muscle tissue was taken from nine wild bird species, DNA isolation was performed using a commercial kit, and the target gene region was amplified by using PCR method. The genetic structure of the species was determined by sequence analysis of the obtained target PCR products and a phylogenetic tree was drawn. The results were evaluated at haplotype level by comparing with the sequences in the gene bank. It was determined that the haplotypes determined for *Tyto alba*, *Ciconia ciconia*, and *Elanus caeruleus* species were new and not found in other countries.

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Introduction

The dominance of steppe vegetation in Şanlıurfa's general vegetation creates suitable habitats for many bird species both for feeding and breeding. In addition, the formation of dam lakes with the construction of dams in recent years has also created different habitats and caused some aquatic bird species to settle in the region. Birds are a species-rich group of vertebrates and are unique in their adaptation to a wide variety of habitats [1]. Birds are generally key species in ecosystems as herbivores, predators, scavengers, seed dispersers and pollinators [1]. Therefore, biodiversity and population density are used as indicators to assess the root causes of biological diversity changes in ecosystems

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around the world [3,4]. The rapid increase in the human population causes changes in the world and as this change causes a decrease in the feeding and breeding areas of many species, it can cause the populations belong these species to decrease or even disappear [1]. Although wild animals are protected by special laws, their numbers continue to decrease day by day. Bird species are threatened, as are many species, and 24% of all bird species are listed under the category of concern in the IUCN Red List [1]. Therefore, it is necessary to take precautions by investigating their phylogenetic origins and migration routes. Phylogenetic relationships of birds help to better understand the evolution of important anatomical and ecological traits [6].

Mitochondrial DNA (mtDNA) is a very important tool in evolutionary and population genetics, including molecular ecology, and is commonly used as it provides rich data sources for sequence analysis and genetic diversity and phylogenetic analysis [7,8]. In this study, it was aimed to determine the phylogenetic analyzes of some wild winged bird species found naturally or through migration within the borders of Şanlıurfa by using the mtDNA marker and to compare the sequences in the gene bank to reveal the similarities and differences between them according to NCBI. In addition, it was aimed to create a wider data area for the species by registering different results in the NCBI gene bank.

Material and Methods

No field study was conducted within the scope of the research and no samples were collected from nature, all samples used as materials were made on wild bird species that were brought to Gölpınar Wildlife Rescue and Rehabilitation Center in Şanlıurfa Karaköprü district, which died naturally or were treated but could not be saved. Information about the samples is presented in Table 1. In order to can be made phylogenetic analysis of these samples, research permissions were obtained from the General Directorate of Nature Conservation National Parks, dated 02.08.2022 and numbered E-21264211-288.04-6454461.

For DNA isolation, an average of 1 gram of muscle tissue from each individual was taken by the Veterinarian and transferred to Harran University, Faculty of Science and Letters, Department of Biology, Zoology laboratory on the same day in cold chain.

DNA isolation was obtained from muscle tissue using Commercial Kit (GeneJET, Thermo Scientific). GeneJET and Thermo Scientific commercial kits used for DNA isolation were preferred because they are practical and economical. In order to control the

DNA quality and presence, DNA samples of each individual were loaded into the wells of 0.8% agarose gel with SYBR Green added, and electrophoresis was carried out at 120 Volts for 30 minutes and then visualized in a UV light device (Smart View Pro Imager System, Major Science). The primer pair used for duplication of the mtDNA COI gene region is taken from [9] study (LCO1490: 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3'; HCO2198; 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3').

Table 1 Information on the sample species used in the study

Order	Common name	Scientific name	Locality	Migrants statuses	IUCN Redlist
Gruiformes	Common Crane	*Grus grus*	Şanlıurfa-Akçakale	migrants	LC (Least Concern)
Accipitriformes	Common Buzzard	*Buteo buteo*	Şanlıurfa-Viranşehir	migrants	LC (Least Concern)
Accipitriformes	Eurasian parrowhawk	*Accipiter nisus*	Şanlıurfa-Haliliye	migrants	LC (Least Concern)
Strigiformes	Barn owl	*Tyto alba*	Şanlıurfa-Hilvan	not migrants	LC (Least Concern)
Ciconiiformes	White stork	*Ciconia ciconia*	Şanlıurfa-Siverek	migrants	LC (Least Concern)
Charadriiformes	Eurasian woodcock	*Scolopax rusticola*	Şanlıurfa-Siverek	migrants	LC (Least Concern)
Accipitriformes	Long-legged buzzard	*Buteo rufinus*	Şanlıurfa-Siverek	migrants	LC (Least Concern)
Pelecaniformes	Glossy ibis	*Plegadis falcinellus*	Şanlıurfa-Birecik	migrants	LC (Least Concern)
Accipitriformes	Black-winged kite	*Elanus caeruleus*	Şanlıurfa-Hilvan	not migrants	LC (Least Concern)

In the multiplication reactions with Polymerase Chain Reaction (PCR), the bonding temperature gradient processes of the primers were made in a Thermal Cycler (BIO-RAD T100™). PCR conditions; 3 min. at 95°C first denaturation, 30 sec. at 95°C denaturation, 45 sec. at 50°C connecting and 60 sec. at 72°C elongation, a total of 35 cycles were run, at the end of the samples were ended at keeping 72°C for 10 minutes. In the PCR mixture used for the replication for the target region; It is in the form 13.9 µl of dH_2O, 1x PCR buffer, 2.5mM $MgCl_2$, 1 µl of 0.5 mM primer (F and R), 0.2 mM dNTPs, 0.1 µl of Taq polymerase (the manufacturer should be given) and 90 ng/µl of template DNA, a total of 25 µl. 2% agarose gel was used to control the products formed after PCR. The products were loaded into the wells in the gel and run at 90V electric current for 40 minutes and monitorized on the device that emits ultraviolet light. Then, the PCR products were sent

to a commercial company and sequence analysis was made on the 3500 XL Genetic Analyzer (Thermo Fisher Scientific).

Raw data of mtDNA sequences sent to us from the commercial company were evaluated using the ChromasPro v 2.0.1 (Technelysium Pty Ltd) program and converted to FASTA format. Sequences obtained were checked using BLAST and compared with species in the NCBI (www.ncbi.gov) database. Sequences in FASTA format all sequences were aligned using BioEdit software version 7.2.5. Phylogenetic analyzes between species were carried out in MEGA X program according to the Neighbor joining tree model using the K2 parameter and phylogenetic tree was created [10]. The bootstrap test (1000 replicates) was used to test the reliability of nodes (tree branches).

Results

Sequence analysis of an average of 630 base pairs of mtDNA COI regions was made for nine wild bird species that were sequenced. The chromatogram image of the FinchTV program belonging to the randomly selected *Plegadis falcinellus* species in the analyzes is shown in Figure 1. 175 polymorphic regions were detected in the target gene region of nine species analyzed for sequence analysis, which shows that it can be used in terms of distinguishing species from each other for phylogenetic analysis.

The sequences belonging to the species obtained in the study were aligned and phylogenetic analyzes were made in the MEGA X program and a Neighbor-joining tree was created. In Figure 2, the phylogenetic tree created by Neighbor-joining method using the sequences of the mtDNA COI region obtained in this study is visualized by adding photographs against each species.

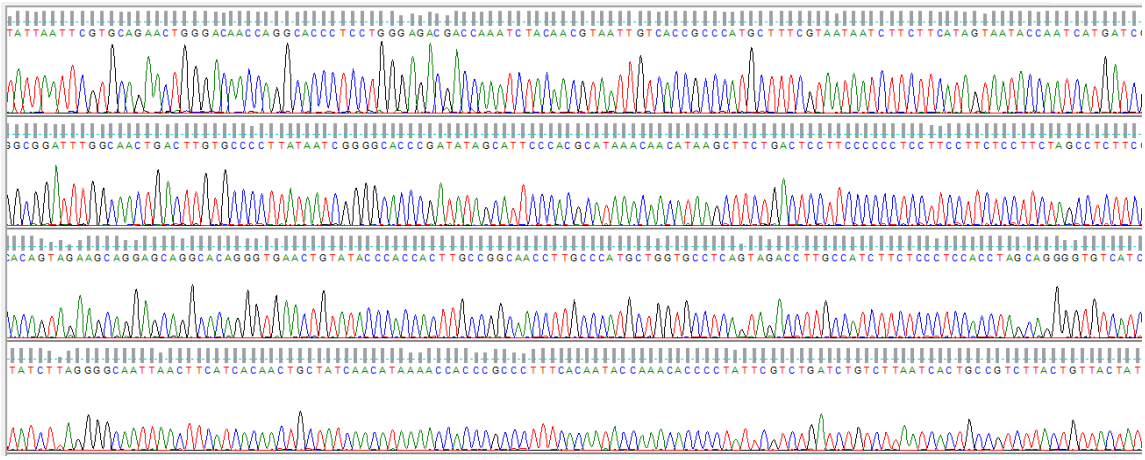

Fig 1 Chromatogram image of a sample sequence analysis of the mtDNA COI region

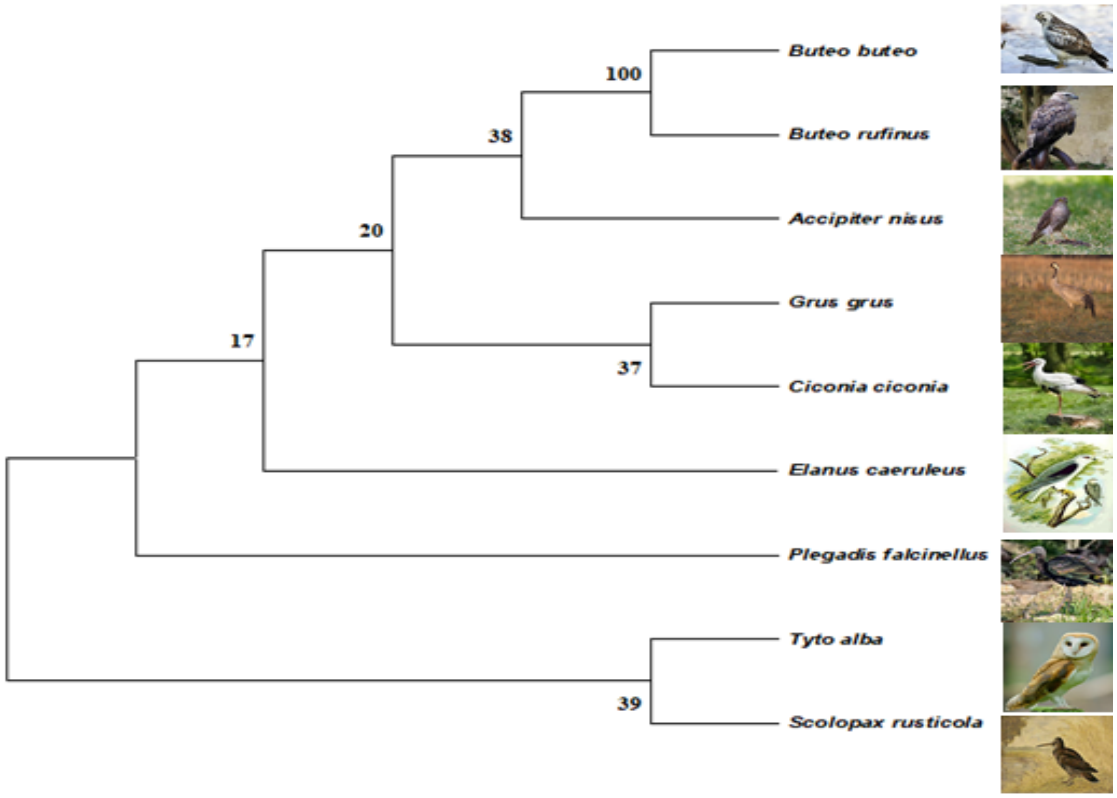

Fig 2 Tree model constructed according to the highest probability method based on mtDNA COI sequences

All of the photos are taken from the website https://www.wikipedia.org. On the phylogenetic tree, two species named *Tyto alba* and *Scolopax rusticola* were separated from the other species by being located on a separate branch. It is seen on the tree that *Buteo buteo* and *Buteo rufinus* species and *Grus grus* and *Ciconia ciconia* species are closely related to each other on the tree. In addition, the genetic distances of the species were calculated to take values between 0.00188 and 0.21137, and it was determined that the closest species to each other were *Buteo buteo* to *Buteo rufinus*, and the most distant species to each other were *Tyto alba* to *Accipiter nisus*.

Discussion

Birds are being affected more and more day by day due to biotic and abiotic factors in natural life. While the damages caused by natural enemies and natural disasters are repaired in time in natural balance, the dangers posed by humans cause the population of many bird species to shrink and their extinction to disappear from the World [11].

In particular, the decrease in individuals in the natural population may cause the disappearance of unique genotypes, and when this genetic information is lost, it is almost impossible to recover it [12,13]. Therefore, measures should be taken for all factors that reduce the genetic diversity of natural species so that the chances of survival of populations increase. Because genetic makeup directly reflects the ability of species or populations to adapt to unfamiliar or regenerated environmental conditions it [14,15].

Table 2 The haplotypes detected in the mtDNA COI gene in this study The similarity rates between the base sequences of the haplotypes in the NCBI database

Species name	Accesion number	Similarity Rate (%)	Country
Grus grus	OQ345516	100	This study
	GU571916	100	Scandinavian countries
	GU571425	99.84	Scandinavian countries
	GU571917	99.68	Scandinavian countries
Buteo buteo	OQ345517	100	This study
	KT803616	100	Cyprus
	GU571769	100	Scandinavian countries
	KY754488	99.81	Central European
Accipiter nisus	OQ345518	100	This study
	GU571689	100	Scandinavian countries
	KF946553	100	Netherlands
	AB843328	100	Japan
Tyto alba	OQ345519	100	This study
	KF946926	99.52	Netherlands
	KF432222	99.67	Netherlands
	GU572154	99.68	Scandinavian countries
Ciconia ciconia	OQ345520	100	This study
	KY754495	99.84	European countries
	GU571816	99.84	Scandinavian countries
Scolopax rusticola	OQ345521	100	This study
	GU572087	100	Scandinavian countries
	AB843147	99.69	Japan
	AB843750	99.53	Japan
Buteo rufinus	OQ345522	100	This study
	KT803615	100	Cyprus
Plegadis falcinellus	OQ345523	100	This study
	GU572048	100	Scandinavian countries
	DQ433121	100	North American
Elanus caeruleus	OQ345524	100	This study
	MK932886	99.84	Thailand
	OK662584	99.84	China

In this study, the genetic structure of some bird species that naturally live in the Şanlıurfa region or use it as a migration route was evaluated phylogenetically by making sequence

analyzes of the mtDNA COI gene region. Sequences of all species obtained in this study were compared with similar sequences in the NCBI gene bank by applying the BLAST technique. The similarity rates between these sequences and information on the countries where the bird species live are given in Table 2.

All genetic data obtained as a result of this study were registered for the first time in the NCBI gene bank for Türkiye and their accession numbers (GenBank OQ345516-OQ345524) were obtained. The haplotypes of *Grus grus*, *Buteo buteo*, *Accipiter nisus*, *Scolopax rusticola*, *Buteo rufinus* and *Plegadis falcinellus* species seen in Şanlıurfa had found to be the same as those seen in other countries. However, for *Tyto alba*, *Ciconia ciconia*, and *Elanus caeruleus* species, the haplotypes detected in this study are seen to be new haplotypes not found in other countries. Due to the rapid increase in the world population, the spread of urbanization, the destruction of forests, agricultural areas and areas such as streams, the habitats of many living species, including birds, are destroyed and their species are endangered. [5]. It is estimated that genetic diversity will decrease faster than species diversity under increasing threats [16]. The discovery of new haplotypes is accepted in some population to be indicative of a unique genetic construction. Therefore, the protection of individuals with unique genotypes is important for the continuation of populations.

Conclusion

Changing ecological conditions will cause to the emergence of new haplotypes over time and increase the adaptation ability of individuals to the environment for the continuation of populations. For this reason, it is necessary to identify the feeding and breeding areas on the migration routes of bird species and to protect these areas without changing their natural characteristics. Taking samples from a large number of individuals from the same species in Şanlıurfa province will be able to better understand the genetic diversity and migration periods of the species. In addition, more comprehensive results can be obtained with studies to be carried out on samples to be obtained in different provinces.

Abbreviations

IUCN: The International Union for Conservation of Nature ; NCBI: The National Center for Biotechnology Information ;DNA: Deoksiribo nukleik acid; BLAST: The Basic Local Alignment Search Tool; mtDNA COI: Mitocondrial Deoksiribo nukleik acid Cytochrome c oxidase ; MEGA X; Molecular Evolutionary Genetics Analysis ;SYBR Green: It is used as a dye for the quantification of double stranded DNA in some methods of quantitive PCR ;UV: ultraviole.

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Compliance with ethical standards
Conflict of interest
The authors declare no conflict of interest.

Ethical standards
The study is proper with ethical standards.

Authors' contributions
In this work, the laboratory works and analyze were conducted by Dr. Arif Parmaksız. Sampling and field work were done by Adil Uztemur. The manuscript was edited and finalised by Dr. Cahit Çeçen

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