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MITOCHONDRIAL DNA ANALYSES OF DOMESTIC PIGEON BREEDS (COLUMBA LIVIA DOMESTICA) IN TURKEY

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

BENGİSU BİRAY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGY

MAY 2020

Approval of the thesis:

THESIS TITLE

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ABSTRACT

MITOCHONDRIAL DNA ANALYSES OF DOMESTIC PIGEON BREEDS (COLUMBA LIVIA DOMESTICA) IN TURKEY

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May 2020, 116 pages

"Domestic pigeon" refers to any human-developed breed originating from its wild relative, the rock pigeon. Domestic pigeons were used as a food source by humans ~10,000 years ago, while Ancient Egyptians used them as both food and ceremonial animals. Today, there are approximately 350 different breeds and thousands of breeders. Many breeds were selected for their flight abilities or for their extreme morphological characteristics. Through intense artificial selection, pigeon breeds were subjected to a massive directional selection that gave rise to great phenotypic diversity among them. In this study, feathers of different breeds (particularly "owl" breeds) collected from Turkish and foreign breeders were used as DNA source to study their phylogenetic structure. Two mitochondrial regions, *COI* and D loop, were used to analyze divergence and possible phylogenetic links. The *COI* marker did not provide enough data to infer reliable construction of pigeon breed phylogeny. The D loop marker did not differentiate between major groups, either, except for the Modern Oriental breed. A star shaped Median-Joining Network suggested little or no geographical structure. Low level of sequence divergence and the high frequency

of unique mutations indicated rapid population expansion. Our findings confirm the probable single origin of all domestic breeds and the widespread practice of indiscriminate crossbreeding between various breeds. Overall, mtDNA turned out to be an uninformative marker for a reliable domestic pigeon phylogeny, and was unable to shed light to even well documented events such as the introduction of the Hünkari breed into Europe in the 19th century.

Keywords: Columba livia domestica, mtDNA, *COI*, D loop, Phylogeny

ÖZ

TÜRKİYEDEKİ EVCİL GÜVERCİN SOYLARININ (COLUMBA LIVIA DOMESTICA) MİTOKONDRİYAL DNA ANALİZİ

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Mayıs 2020, 116 sayfa

"Evcil güvercin" *Columba livia*'nın insanlar tarafından geliştirilmiş farklı soylarını betimlemektedir. Evcil güvercinler yaklaşık 10,000 yıl önce Bereketli Hilal bölgesindeki insanlar tarafından besin kaynağı olarak kullanılmış, Antik Mısırlılar da güvercinleri hem besin kaynağı hem de ritüel hayvanları olarak kullanmışlardır. Günümüzde, yaklaşık 350 farklı evcil güvercin soyu ve birçok yetiştirici bulunmaktadır. Soylar, uçuş ile ilgili kabiliyetleri ya da farklı morfolojik özellikleri bakımından seçilmiştir. Bu kuvvetli yapay seçilim, güvercin soylarının, soylar arasında son derece fazla fenotipik çeşitliliğe sebebiyet veren kuvvetli bir yönlü seçilime maruz kalmasına sebep olmuştur. Bu çalışmada, yerli ve yabancı yetiştiricilerden toplanan farklı evcil güvercin soylarının (özellikle "owl" tipi soyların) tüyleri popülasyonun filogenetik yapısını incelemek üzere DNA kaynağı olarak kullanılmıştır. İki mitokondriyal bölge, *COI* ve D loop, soylar arasındaki farklılaşmayı ve olası filogenetik bağlantıları analiz etmek için kullanılmıştır. *COI* markeri, güvercin ırkları için güvenilir bir filogeni sunma konusunda yeterli veriyi sağlamamıştır. D loop markerı da Modern Hünkari soyu hariç, büyük soy grupları arasında ayrım yapamamıştır. Yıldız şeklindeki Median-Joining Ağı, popülasyonda

coğrafi yapının çok az olduğunu veya hiç olmadığını gösterirken, düşük seviyedeki sekans ayrışması ile farklı mutasyonların sık görülmesi ani gerçekleşmiş olan bir popülasyon genişlemesine işaret etmektedir. Bulgularımız, tüm evcil soyların tek bir olası kökeni olduğunu ve çeşitli soylar arasında ayrım gözetmeden yapılan çaprazlama uygulamalarının yaygın olduğunu doğrulamaktadır ancak bunu, muhtemelen, söz konusu soy için istenen özellikler bakımından uygulanacak seçilim izleyecektir. Özetle, mtDNA, yerli güvercin soylarının filogenisini oluşturma konusunda yeterli bilgiyi sağlayamamaktadır ve Hünkari ırkının 19. yüzyılda Avrupa'ya götürülüşü gibi iyi belgelenmiş olaylara bile ışık tutmakta yetersiz kalmıştır.

Anahtar Kelimeler: Columba livia domestica, mtDNA, *COI*, D loop, Filogeni

To my mother and grandmother

ACKNOWLEDGMENTS

I want to thank to my advisor Prof. Dr. C. Can Bilgin and my co-advisor Assist. Prof. Dr. Çiğdem Akın Pekşen for their precious contributions they provided during the whole thesis process.

Also, I would like to thank Prof. Dr. Murat Türkeş and Serkan Gündüz, who did not refrain to support me all the time and patiently answered my timeless questions.

Special thanks to all of the breeders who spent their valuable time to provide me bird feathers; Erkan Savran, Sırrı Yıldırım, Muhammet Çırak, A. İhsan Otamış, İskender Damgacı, Hüseyin Eroğlu, Serkan Gündüz, Muzaffer Göl, Celal Erhan Barut, Ömer Bozkuş, Mutlu Helvacı, Ahmet Cila, Erol Osmanlı, Ali Arslan, Ümit Ertürk, Ekrem Çavuş, Mehmet Demir, Hüseyin Zabun, Ersoy Koldaş, Sertaç Adabay, İsa Savaşkan, Murat Türkeş, Mustafa Mert Yeşilkaya, Ramazan Özdemir, Ahmet Oyar, Muzaffer Bey, Yasir Arabacı, Ahmet Yağız, Mustafa Kuzucuk

Thank you, Alexandra Elbakyan, the woman who made all of this possible by removing the obstacles to the open science.

Finally, thanks to my family and friends for their unconditional love, even when I was the most unbearable.

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LIST OF ABBREVIATIONS

ABBREVIATIONS

Base pairs: bp(s)

Before Christ: BC

Bayesian Information Criterion: BIC

Centimeter: cm

Cytochrome c oxidase subunit I: *COI*

Deoxyribonucleic acid: DNA

Distilled Water: dH2O

Displacement loop: D loop

Dithiothreitol: DTT

EPH receptor B2: EphB2

Hasegawa-Kishino-Yano: HKY

International Union for Conservation of Nature: IUCN

Kilometer: km

Maximum Likelihood: ML

Melanocortin 1 receptor: MC1R

Microliter: µl

Milliliter: ml

Mitochondrial DNA: mtDNA

Molar: M

Molecular Evolutionary Genetics Analysis: MEGA

National Center for Biotechnology Information: NCBI

Neighbor Joining: NJ

Nanometer: nm

Numts: Nuclear Mitochondrial Sequences

PCR: Polymerase Chain Reaction

Paired-like homeodomain 1: Pitx1

Ribonucleic acid: RNA

Revolutions per minute: rpm

Solute Carrier Family 45 Member 2: Slc45a2

Single Nucleotide Polymorphism: SNP

Sry-Box Transcription Factor 10: Sox10

Short Tandem Repeat: STR

Standard Neutral Model: SNM

Tyrosinase Related Protein 1: Tyrp1

T-Box Transcription Factor 5: Tbx5

Variable Number Tandem Repeat: VNTR

Ultra Violet: UV

LIST OF SYMBOLS

SYMBOLS

°C: Celsius

µl: microliter

CHAPTER 1

INTRODUCTION

1.1 Taxonomy of the Rock Pigeon, *Columba livia*

1.1.1 The rock Pigeon, *Columba livia* (Gmeiln, 1789)

Rock pigeon (*Columba livia*) is a member of the *Columba* genus which is located in Columbidae family of Aves class (BirdLife International, 2019).

1.1.2 The *Columbidae* Family

Pigeons and doves are found everywhere on Earth except Antarctica. Except a few species they constitute a morphologically uniform family. This family can live everywhere except extremely hot and cold environments. 316 species of pigeons and doves are known up to now (Gibbs, Eustace, & Cox, 2001).

This family can be divided in five different sub-families (Gibbs et al., 2001):

i) *Columbinae* (typical seed-eating pigeons: 187 species)

ii) *Otidiphabinae* (Pheasant Pigeon: 1 species)

iii) *Treroninae* (fruit doves: 124 species)

iv) *Gourinae* (crowned pigeons: 3 species)

v) *Didunculinae* (Tooth-billed Pigeon: 1 species)

1.1.3 *Columba*

Columba is the largest genus of this family, including 54 species. This genus is close relative of the genus *Streptopelia*. Relationship of *Columba* with the other genera is not fully understood. The word Columba is used to refer rock pigeons (*olivae, eversmanni, oenas, rupestris, livia*) mainly represented by the *livia*. *Columba livia* is the most known and largely distributed member of the family, including lots of different feral and domesticated variety (Gibbs et al., 2001).

1.2 Description of the Rock Pigeon

Wild Rock Pigeons have blue-grey color and green-purple gloss behind their dark colored neck. They can be easily distinguished from many of the sympatric species by their relatively short tail and long wings, pale medium-grey feathers, black bands found on their secondary feathers, black band on the end of their tail and their white underwings. A medium-sized *Columba livia* is usually smaller than the Common Wood Pigeon (*Columba palumbus*) and larger than the Stock Pigeon (*Columba oenas*) (Gibbs et al., 2001).

Some of the domesticated pigeons have escaped from their home in time and have hybridized with the wild pigeons and thus constructed feral pigeon populations (Sol, 2008). Rock pigeons established the first feral populations in North America 400 years ago (Shapiro & Domyan, 2013). Feral pigeons can be found nearly everywhere on earth ("Pigeon," 2017). Due to the ongoing natural selection, feral pigeons have become morphologically similar to the Wild Rock Pigeons of Old World and have lost the phenotypic variation which domestic breeds have (Sol, 2008) (Shapiro & Domyan, 2013). Similar phenotypes have begun to be observed in different feral populations lost the genetic diversity of their domestic ancestors. And natural selection has led to the re-emergence of the size and shape of wild rock pigeons instead of the domestic ancestors of feral populations (Sol, 2008).

Feral Rock pigeons have a grey body with whitish rump, black lines on their secondary, broadband on their tail and red feet and longer wings than the wild rock pigeons have. Their plumage patterns are rarely symmetrical (Phillips, 2008) (Gibbs et al., 2001). As domestic pigeon races are developed for lots of different kinds of features like appearance, endurance, racing abilities and meat yield by breeders, they show a great level of phenotypic variation among breeds (Shapiro & Domyan, 2013) (Joint Working Group on Refinement, 2001).

Their height is 31-34 cm, their body weights are between 243-359 grams and their wings are 63-70 cm. When taking off, the wing tips touch each other and produce a characteristic sound (Phillips, 2008).

Their common call is an “oorh” or an “oh-oo-oor”, resembling groaning. They produce an “oo-roo-coo t’oo” sound with one second intervals when they are displaying. The stress call is usually an “oorh”. The males generally sing more than the females (Gibbs et al., 2001).

1.3 Distribution and Habitat

The original distribution of this species is difficult to determine because of the long history of the species and intensive domestication process (Gibbs et al., 2001) (Shapiro & Domyan, 2013). They are thought to have spread to the world from Europe, West Asia, North Africa and the Middle East (Phillips, 2008) (Shapiro & Domyan, 2013) (Gibbs et al., 2001). Their spread has been carried out as a source of food or as pet. Some of the birds raised for hobby purposes have lost and not returned home and have contributed to the establishment of new populations (Gibbs et al., 2001). They do not migrate, but can fly daily at a distance of up to 50 km from their home or breeding sites (Phillips, 2008). Worldwide distribution of native and introduced *Columba livia* populations is shown in Figure 1-1.

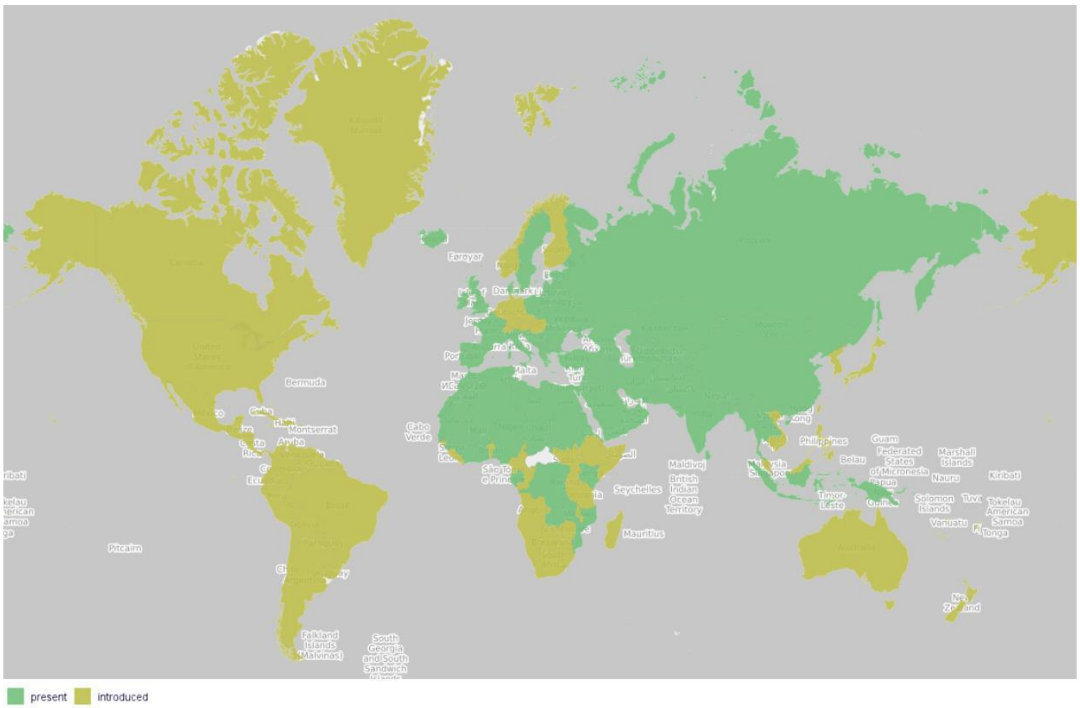

Figure 1-1 Worldwide distribution of *Columba livia.* (“Rock Pigeon,” 2019).

They are commonly found in cliffs, valleys, mountainous areas, caves and rocks. The maximum altitude they can survive is about 4500 meters (Gibbs et al., 2001) (BirdLife International, 2019). They prefer openness and tree-free areas, Mediterranean steppes, agricultural areas and deserts (Gibbs et al., 2001) (Joint Working Group on Refinement, 2001).

Feral ones are found in agricultural areas, rural areas or cities. Since they also feed with human waste (scavengers), they prefer areas where people live as habitats, but lost or stray racing pigeons can be found everywhere (BirdLife International, 2019), (Gibbs et al., 2001) (Phillips, 2008). Pigeons are usually raised by breeders for hobby purposes, but they sometimes can be fed to be used as food source, too (Phillips, 2008).

1.4 Biology and Ecology

1.4.1 Nutrition

They generally feed on seeds, grains, plants, sometimes green leaves, buds and rarely invertebrates. They feed in rural areas where seeds of plants such as corn and wheat are found. When winter comes and the surface is covered with snow, they prefer to be fed near the storage areas where these seeds can be poured. They look for food on the ground with the typical movements of moving their heads back and forth. If it is present, they also prefer high-protein foods such as peas (Phillips, 2008) (Joint Working Group on Refinement, 2001) (Gibbs et al., 2001) (Hume, 2002).

1.4.2 Sex Determination

Pigeons generally are sexually monomorphic but males of some breeds may show remarkable phenotypical features such as color or pattern. Males may be more structured. Their heads are more rounded, ceres and wattles are larger than females. Pigeon sexing is usually done by observing their behavior. Males produce double “coo” sound, move up and down while swaggering. Females usually are not bulky as males, have a flatter head and produce a single “coo” sound (Sol, 2008) (Joint Working Group on Refinement, 2001). Example photographs of female and male pigeons are shown in Figure 1-2 and Figure 1-3.

Figure 1-2 A pair of pigeons. The pigeon on the right is the female and the pigeon on the left is the male. The birds in the photo belong to the "Malatya Bolpacha" breed. This photograph was taken by Bengisu Biray on 1 April 2019.

Figure 1-3 Female and male feral pigeons. (Smith, 2013).

1.4.3 Mating Behavior

They are found as couples in breeding season (Joint Working Group on Refinement, 2001) ("Pigeon," 2017). They can be found as groups in their natural habitats, while they are foraging and perching together. They are extremely territorial, they defend their nesting and perching areas (Joint Working Group on Refinement, 2001) (Mcgregor & Haselgrove, 2010). While the wild ones are usually present in small groups, the feral pigeon groups are more crowded (Gibbs et al., 2001). When they are present as crowded groups, aggressive competition for food and partner can be seen (Mcgregor & Haselgrove, 2010).

They exhibit different courtship behaviors. The male flaps wings and perches next to the female which is calling and leaping into the air as the male approaches him. When he is located in close proximity, he fluffs his feathers out, raises his head while his beak pointing down and spreads and suppresses his tail. He inclines, quickly circles, lifts its head, and turns back. In another courtship behavior, he turns around the female again and again, while his head is down (Gibbs et al., 2001) (The Cornell Lab of Ornithology, n.d.). The male can vomit into the female's beak or they can preen each other's feathers (The Cornell Lab of Ornithology, n.d.). When threatened, the male approaching to the rival, lowers his head and sings a confident "oo roo-coo-t'coo" call (Gibbs et al., 2001).

1.4.4 Nesting and Reproduction

Pigeons nest to cliffs, caves, steep valleys and deep wells (Gibbs et al., 2001). The nest material is provided by the male, it also takes care of the nest and the female (Phillips, 2008). The nest is loosely formed from roots, twigs and leaves (Hume, 2002) (Gibbs et al., 2001). The male brings a branch or root to the nest and the female builds the nest with these materials (The Cornell Lab of Ornithology, n.d.). They nest to a rock ledge, cave, or to a deep cleft on the rocky face of the cave. The feral

ones usually nest in the parts of man-made buildings that resemble rocks in nature (Gibbs et al., 2001).

They are usually monogamous (Gibbs et al., 2001) (Joint Working Group on Refinement, 2001). Males remove the female from the group during the breeding season. If the female's partner fails to mate or dies, the female can mate with another male (Joint Working Group on Refinement, 2001). They can reproduce throughout the year.

1.4.5 Life Cycle

They usually make 2 eggs, but sometimes they can only lay one elliptical egg. A Rock Pigeon nest including two eggs and two nestlings are shown in Figure 1-4 and Figure 1-5 respectively. The incubation period starts 8 to 12 days after mating and lasts 16 to 19 days (Gibbs et al., 2001) (Phillips, 2008). The male and female pigeon incubates the eggs respectively (The Cornell Lab of Ornithology, n.d.). Both the male and female feed their offspring with a highly nutrient-containing liquid, crop milk, secreted from their crops, mostly consists of fat and protein (Gibbs et al., 2001) (Phillips, 2008) (The Cornell Lab of Ornithology, n.d.) (Sales & Janssens, 2003). Crop milk may also contain antibodies which give strength to the young to fight against parasites and microbes, and also improve the survival of the offspring (Kaplan & Rogers, 2001). Nestlings begin to feather at the 4th or 6th week and reach sexual maturity after 6 months. Domestic pigeons live up to 15 years, while the average lifespan of street pigeons in the city is 3 to 4 years. (Phillips, 2008) (BirdLife International, 2019).

Figure 1-4 *Columba livia* nest with two eggs. (Caffi, 2013a).

Figure 1-5 *Columba livia* nest with two nestlings. (Caffi, 2013b).

1.5 Conservation Status

According to the evaluation report published by Avian Conservation Assessment Database in 2017, predicted size of the global population was 150,000,000 (Avian Conservation Assessment Database, 2017) and according to the report published in

2019, it was about 140,000,000 individuals (Avian Conservation Assessment Database, 2019). The global population size according to IUCN is 260,000,000 (BirdLife International, 2019) on the basis of the 2004 North American Land Bird Conservation Plan (Rich, et al., 2004). In light of these data, it can be said that population size is decreasing. This decline is thought to be due to domestication activities (BirdLife International, 2019). The habitat of the species is substantially large and the number of individuals in the population is quite high, so it does not have the criteria to be classified as *vulnerable*. Although population size appears to be declining, considering population trend, this decline is not fast enough to move the population to the vulnerable status. For this reason, they are classified as *Least Concern* (BirdLife International, 2019).

1.6 Economic Importance

Breeding pigeons for meat have developed mostly in America and Europe (Mead, 2005). Commercial pigeon breeding began in North America in the 1900s. According to Aggrey and Cheng, annual squab production in the United States and Canada in the 1990s was more than half a million tons (Aggrey & Cheng, 2009). Only very young birds called squab are produced and used for the pigeon meat market before their meat becomes harder (Mead, 2005) (Jerolmack, 2007). Squabs, consumed as food, are not fed with anything except pigeon milk and they are not allowed to fly. Pigeon breeds raised for food are called as utility pigeons and generally White King and Red Carneu breeds are preferred (Bolla, 2007). It is advantageous for breeders that pigeons do not easily compete with other animals in the area and adapt quickly to different environments (van der Hoek, Schiere, & Food and Agriculture Organization of the United Nations, 2001). In the optimum conditions, a couple of pigeons are expected to produce 14 - 15 squab per year (Mead, 2005). Although they are easy to produce and grow, they are rarely considered as a source of food (van der Hoek et al., 2001). Today, pigeon meat is mostly served as a luxury meal at finest restaurants (Blechman, 2007).

Nowadays, pigeons are generally raised for hobby purposes. There are approximately 350 different pigeon breeds. These breeds are grown according to their appearance, flight performance or ability to somersault (Shapiro & Domyan, 2013). Today, thousands of pigeon breeders are gathered under various federations and organize pigeon contests in different parts of the world (i.e. the Royal Pigeon Racing Association, the National Pigeon Association, the Federation of National Colombian Pigeon Union, the Irish National Fancy Pigeon Association, etc.). These breeders ensure the protection of this valuable pool of genetic diversity through their activities (Stringham et al., 2012). Breeders have led to the development of a market around this hobby by selling pigeons and buying or selling materials related to breeding.

Rock pigeons can also be used for laboratory research. They are used in behavioral and psychological studies that focus on vision and learning, as they can fulfill the assigned tasks (Stephan & Bugnyar, 2013). They are also used in studies on toxicology (Spears, Brown, & Hester, 2008), physiology (Gumus et al., 2015), pathology (Levenson, Krupinski, Navarro, & Wasserman, 2015) and treatment of avian diseases (Joint Working Group on Refinement, 2001). Additionally, due to their ability to navigate, they are frequently used in the basic researches of bird migration (Chernetsov, 2016).

1.7 Domestic Pigeons

Rock Pigeons are thought to have originated in Europe, North Africa, the Middle East and South Asia. The relationship of humans with pigeons probably dates back to the times when people lived in caves. Caves and rocks were the natural habitats of pigeons. Even in this period, pigeons may have benefited from the food leftovers of humans. Likewise, when people catch them, they may have consumed pigeons as food (Blechman, 2007).

The earliest evidence for the use of pigeons by human communities has been found in the Gorham's Cave. In the 2014 study of Blasco et al., it was found that Neanderthals (and subsequently modern humans) have regularly used rock pigeons as a food source. The rock pigeon bones coming out of the cave were dated to 28 thousand years and 67 thousand years ago. Evidence from Bolomor Cave and Lazaret Cave dates the use of pigeons up to the Middle Pleistocene period, but these do not seem to be continuous events, as observed in Gorham's Cave (Blasco et al., 2014). Twelve-thousand-year-old pigeon bones also found in a cave used as a human settlement in Israel also indicate that ancient hunter-gatherers have used wild pigeons as a source of food. (Jerolmack, 2007).

When people domesticated seeds for farming, they may have also begun to domesticate pigeons without knowing it. Grains spilled on the ground at the time of harvest may have become an easy-to-reach food source for pigeons. Human settlements also have been suitable nesting areas for pigeons. All these factors have helped to further the relationship between humans and pigeons. (Blechman, 2007) (Jerolmack, 2007). It is thought that the domestication of pigeons began in the Fertile Crescent region during the Neolithic times (Driscoll, Macdonald, & O'Brien, 2009) (Shapiro & Domyan, 2013) (Vickrey, Domyan, Horvath, & Shapiro, 2015). Pigeons are the first domesticated species of birds among the domesticated animals (Jerolmack, 2007).

There are several important artifacts informing us about the past of human - pigeon relationship. Pigeon figures can be seen in Egyptian reliefs dated to 2700 BC. A terra-cotta, dated 5th century BC, having pigeon figures carved on, and a tombstone dated to 500 BC, depicting a man holding pigeons in both hands, were found in Turkey and Greece respectively (Jerolmack, 2007). In addition, a pigeon was painted in a ring on a *COI*n belonging to the ancient Greek city of Scyon (400-370 BC). On the bronze *COI*ns found in Apameia (a Phrygian city), the pigeon mentioned in Noah's story is depicted flying over the ark (133–48 BC) (Arnott, 2007). Based on these historical artifacts, it can be said that the human - pigeon relationship dates back long enough for pigeons to have a place in human culture.

From Homer (8th century BC), Socrates and Aristotle (384 – 322 BC), we can see that the Greeks have general information about pigeons as well as selective breeding. There are also records dating back to 200 BC that the Romans used pigeons as a source of food. Pigeons also have an important place in mythology and literature (Jerolmack, 2007) (Arnott, 2007).

One of the most common uses of pigeons is transferring messages. It is thought that the first people to recognize and use the homing abilities of pigeons were Mediterranean sailors. When pigeons are released from the open sea, they go directly to the nearest land to find food, and sailors follow them to land. In time, people began to use this ability of pigeons by manipulating it, and transmit messages (Blechman, 2007). The Egyptians, who used the pigeons to announce the ascendance of a new Pharaoh and probably to report the water level changes in Nile, could be the first community to use this bird as a messenger (Blechman, 2007). In an Egyptian relief dating back to 1350 BC, it is shown that the pigeons were released from their cages and then returned their home (Jerolmack, 2007). Another information about a pigeon carrying a message belongs to 444 BC, the winner of the Olympics was reported by the message carried by a pigeon. Alexander the Great (356 – 323 BC) and Hannibal (247 – 108 BC) also used pigeons to coordinate wars and to announce victories (Jerolmack, 2007). According to the Roman bureaucrat Frontius (30 – 130 AD), Decimus Brutus (83 – 45 BC) and his advisor Histius had communicated through pigeons (Mynott, 2018). Genghis Khan and his grandson Kublai Khan, who lived in the 13th century, established a pigeon mail organization covering one-sixth of the world and the sultans of the Persian Empire built pigeon houses for the pigeons they used as messengers (Blechman, 2007) (Jerolmack, 2007).

In “On Agriculture” which has been written in 37 BC, Varro mentions that the most of the people were raising pigeons only for pleasure, not for eating (Jerolmack, 2007). About the breeding of pigeons for hobby purposes, Pliny, has written about pigeon enthusiasts who build pigeon-houses on their rooftops to raise birds and interested with the pedigree and origins of the birds (Kakish, 2012). According to these, people were breeding pigeons for hobby purposes, even in 37 BC.

Much of the information on the history of pigeon breeding for hobby purposes is based on the books written in Europe in the 1800s. Some of the ancestors of modern breeds, such as Fantail (an fancy pigeon breed with a long and fancy tail (Naether, 1939)) and Runt (a pigeon breed raised for both display and meat (Whitman, 1889)) (Naether, 1939)) estimated to be domesticated 860 to 200 years ago, respectively (Shapiro & Domyan, 2013). The ancient geographic source of domestic pigeon diversity is thought to be the Middle East and South Asia. It is known that these birds were exchanged in the 16th century, or even earlier. This indicates possible hybridization among breeds (Shapiro & Domyan, 2013). Yet another possible reason for hybridization is that breeders crossbreed different breeds of pigeons in order to transfer a characteristic (e.g. color or pattern) or to "improve" their breed. This intensity of introgression and hybridization is an obstacle to the exact determination of the time and place of domestication of pigeons (Shapiro & Domyan, 2013) (Stringham et al., 2012). Intense selection operating on pigeons cause extreme differentiation of breed phenotypes from each other. Breeds show great variation in feather colors and patterns, feather placement, sound, flight behavior and many other characteristics (Shapiro & Domyan, 2013) (Stringham et al., 2012) (Vickrey, Domyan, Horvath, & Shapiro, 2015). As can be said for other pets (cats, dogs, chickens), it can be said for also pigeons that the existing genetic variation is the result of a long-running selection experiment. The domestication process begins with a very intensive selection to achieve the desired phenotype. This selection is followed by stabilizing or purifying selection. This process led to the pigeons be the bird with the greatest phenotypic diversity among all bird species (Shapiro & Domyan, 2013). In order to emphasize that the domestic pigeon breeds are very different from each other in morphological terms, Darwin had said that if a taxonomist makes a classification solely on the basis of morphology, he can group them as different genera (Stringham et al., 2012) (Domyan & Shapiro, 2017). Darwin himself, as a pigeon enthusiast and a member of pigeon clubs, had devoted two chapters to pigeons in his book “In Variation of Animals and Plants under Domestication”. Darwin had observed that, no matter how different they appear,

pigeon breeds could produce fertile offspring with each other, and to this respect, he had concluded that all domestic pigeon breeds were variants of a single species, the Rock Pigeon, *Columba livia* (Domyan & Shapiro, 2017). Pigeon breeds has been subjected to artificial selection in many aspects.

Three of the most preferred ones of these features are color/pattern, head crest and feathered feet. A review study conducted by Domyan and Shapiro in 2017, includes information about which genes are responsible from expression of these traits (Domyan & Shapiro, 2017).

1.7.1 Head Crest

Head crest is preferred by breeders in domestic birds and sexually chosen by females in nature. Different types of head crests are shown in Figure 1-6. Shapiro et. al discovered a mutation encoded by a single gene which is found in the highly conserved kinase domain *EphB2*. They found that this mutation is connected with the head crest formed of reverse growth of the feathers on the head (Shapiro et al., 2013) (Domyan & Shapiro, 2017).

Figure 1-6 Different types of head crests. (Shapiro et al., 2013).

In the review of Baptista et al., (2009), it has been mentioned that the morphological traits chosen by humans can also be found in extant irrelevant breeds. These traits may be due to the genes that ancestral *Columbiformes* have had once. These genes suppressed over time, may become active again as a result of both natural and artificial selection (Baptista, Gomez, & Horblit, 2009). This foresight has been

approved by the results of the study conducted by Vickrey et al. (2015). *EphB2* gene has been suppressed in another domestic pigeon, *Streptopelia risoria*, just like in *Columba livia* (Vickrey, Domyan, Horvath, & Shapiro, 2015).

1.7.2 Color and Pattern

Inheritance of feather color is based on the same pigmentation system in pigeons as in mammals. Changes in the amounts of black eumelanin and red pheomelanin can result in the formation of a large proportion of the many color variations that pigeons have. According to the classical studies, locus B, considered to be the basic color locus, is sex-dependent and has three different alleles. These alleles; wild type allele (B+), producing the black eumelanin molecule, dominant ash-red allele (BA), producing pheomelanin and recessive brown allele (b) producing eumelanin (Domyan & Shapiro, 2017). Domyan et al. (2014) have discovered protein coding and cis-regulator mutations in *Tryp1, Sox10* and *Slc45a2* genes which are responsible from the color phenotypes in pigeons. *Tyrp1* gene has been identified as B locus mentioned in the classical studies. The same *Tyrp1* haplotype was observed in all ash-red colored birds examined, indicating that the ash-red color was transferred to different breeds with hybridization or introgression. Other findings of Domyan et al. 2014 are that the recessive red color is caused by a mutation in the *Sox10* gene, which secretes one of the transcriptional activators of the *Tyrp1* gene, and a mutation in the *Slc45a2* gene is associated with light feather color (Domyan & Shapiro, 2017) (Domyan et al., 2014). A scheme of different color and pattern types of pigeons is shown in Figure 1-7.

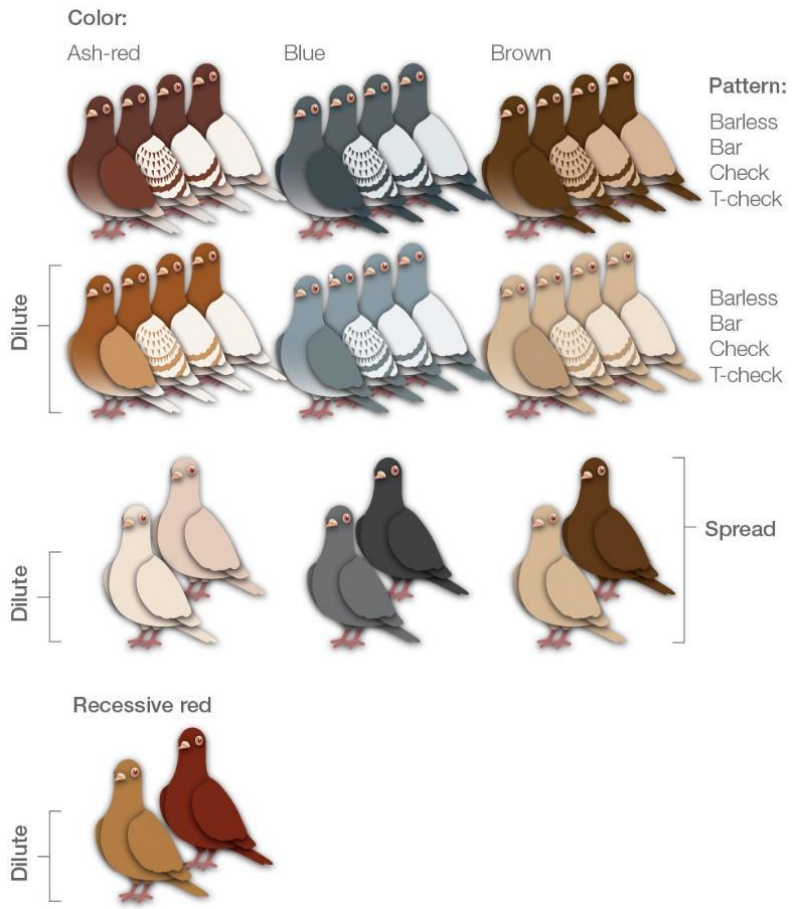

Figure 1-7 Different types of colors and patterns of pigeons. (Domyan et al., 2014).

Studies of the *MC1R* gene, another extensively studied pigmentation gene, showed that it has no relation with different color types in pigeons. (Derelle et al., 2013). In a consecutive study, it was, however, found that *Val85Met* substitution in *MC1R* may be associated with pheomelanin production (red color) in pigeons (Guernsey et al., 2013) (Domyan & Shapiro, 2017). Different combinations of the genotypes *Tyrp1*, *Sox10*, and *Slc45a2* allow the formation of a variety of color and pattern variations in pigeons (Domyan & Shapiro, 2017).

1.7.3 Feathered Feet

Although classical studies suggested that the epidermal attachment type is determined by a small number of loci, the findings of Domyan et al. (2016) study

revealed that this feature has a more fundamental developmental basis (Domyan et al., 2016). In connection with his observations of pigeon breeds, Darwin said that the outer toes of pigeons with feathered feet are often integrated into the skin, meaning an anatomical change of the pigeon feet and Domyan et al. also detected an anatomical change of the feet of feathered feet pigeons. They indicated that the first toe of a pigeon breed with feathered feet stretched forward instead of curling back like the other pigeons (Domyan et al., 2016).

Figure 1-8 Feathered feet examples. (Domyan et al., 2016).

When the whole genome was scanned for allele frequency changes, two loci were found to be responsible for the transition from scaly feet to feathered feet. The first is *Pitx1*, a transcription factor that has an important role in hindfoot morphology. The second is *Tbx5*, also a transcription factor that plays an important role in forefoot development (Domyan et al., 2016). Different types of feathered feet are shown in Figure 1-8.

1.8 Pigeon Breeding in Turkey

The result of archaeological studies in Turkey indicates that findings of historical artifacts belonging to the older Anatolian civilizations have pigeon drawings or embroideries. These artifacts point out that pigeon had symbolic importance for this geography and pigeon breeding have been done for a long time (Yılmaz & Boz,

2012) (İşcen, n.d.). In Turkish culture, the symbol of pigeon had been used in many works, stories, fairy tales and legends. And for some of them, it had been the symbol of fundamental and important entities such as death or soul. Further, due to this spiritual symbolic role, it has not been preferred by the public as a food source (Yılmaz & Boz, 2012). According to the Ottoman archive records, however, pigeons had been used as food in the palace. It is also known that different pigeon breeds had been actively raised in Ottoman palaces (Yılmaz & Boz, 2012). In addition, archival documents showed that there was postal pigeon trade with Russia during the Ottoman period (Yılmaz & Boz, 2012).

Today the breeders come together under the umbrella of Turkey Pigeon Federation. They organize various competitions with respect to the appearance or the flight performances of pigeon breeds. Photographs from different organizations are shown in Figures 1-9, 1-10 and 1-11. There are nearly 50 associations that are members of the Federation ("Türkiye Güvercin Federasyonu," n.d.). In addition to the Federation, there is the Pigeon Breeders Association, which is active for breeders to communicate with each other and exchange information. The Pigeon Breeders Association has a website where breeders can access the information about the distribution, general characteristics, and pigeon diseases of the local breeds raised in Turkey ("Güvercin Birliği," n.d.).

Figure 1-9 5th Oriental Frill Beauty Contest, 2019, Manisa, Salihli ("5th Oriental Frill Beauty Contest," 2019a).

Figure 1-10 Winner of the 5th Oriental Frill Beauty Contest, 2019, Manisa, Salihli ("5th Oriental Frill Beauty Contest," 2019b)

Figure 1-11 Pigeon Beauty Contest for Kids, 2019, Manisa, Salihli ("Pigeon Beauty Contest for Kids," 2019).

Pigeon breeding in Turkey faces many different problems that need to be solved. Although they exist, many associations function as entertainment venues where leisure activities are performed instead of pigeon breeding. Unconscious crossing of different breeds by breeders causes the pure characteristics of the breeds to deteriorate or disappear (Yılmaz & Boz, 2012) (Nurullah, n.d.). Scientific studies on the identification of breeds or their description are also extremely lacking. In addition, pigeon breeders complain about the lack of veterinarians who have knowledge about species and able to diagnose and treat the pigeon diseases correctly (Yılmaz & Boz, 2012).

1.8.1 Turkish Oriental Frill

One of the descendants of the pigeon, called Manisa Hünkarisi in Turkish, is short beaked and frilled. It has been first raised in the Ottoman Palaces and therefore named as Hünkari (means belonging to the sultan) (Tığ, n.d.) (Cemil, n.d.-a) (Koldaş, n.d.). In J.C. Lyell's Fancy Pigeons book, these pigeons are named as Turkish Frilled Pigeons and characteristics and varieties of this breed are given in detail. A drawing describing the Turkish Frilled pigeons is shown in Figure 1-12. According to the book, H. P. Caridia was the first person who brought these birds to England about

20 years before the book was written (estimated 1860s). In this book, the variants of the breed are given as Satinette, Blondinette, Domino, Yizor and Turbiteen. Satinette and Domino are thought to be the oldest varieties. According to Mr. Caridia, the Satinette variety is quite old. Mr. Caridia had acquired the birds from an 80-year-old priest living in Smyrna. He had learned that his father and grandfather also had raised the same breeds. This shows that these birds had been bred over three generations, that is, they had at least 120 years of history in 1860s (Lyell, 1888).

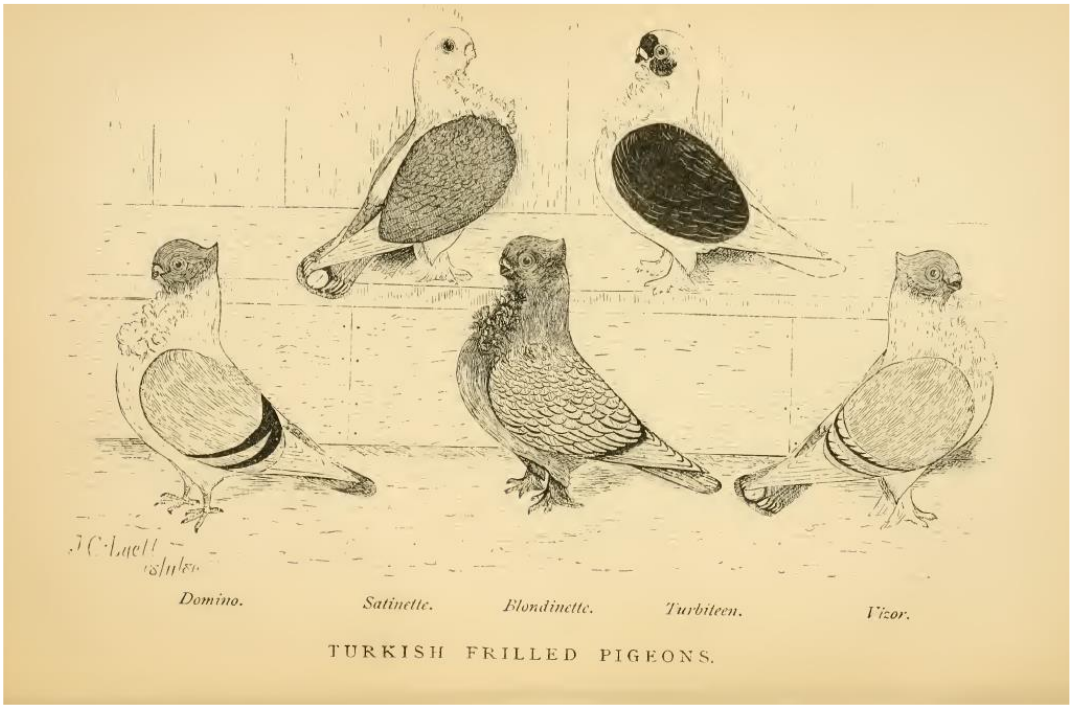

Figure 1-12 A drawing of Turkish Oriental frills form Lyell's Fancy Pigeon book. (Lyell, 1888).

Carl A. Naether refers these pigeons as Oriental Frills, in the "The Book of Pigeon". The book states that the beaks of the old Oriental Frills were longer than the beaks of the present forms. The Hünkari pigeon which was taken from Turkey and improved abroad, is called the “Modern Hünkari” by the Turkish breeders (known as Oriental Frill in general). And the ones that have raised in Turkey are called the “Classical Hünkari”. When compared, the Modern Hünkari breed has very short beak while the traditional Classical Hünkari thought to be native has about half centimeter taller beak than the modern. A photograph of an Oriental Frill pigeon

from Naether's book is shown in Figure 1-13. Photographs of Turkish Oriental Frill (Classical Hünkari) and Modern Hünkari are shown in Figure 1-14 and 1-15 respectively. Considering the statement in the book, the possibility of the breeders' claims to be correct becomes a possibility which is worth to evaluate (Özgür, n.d.) (Cemil, n.d.-b) (Naether, 1939).

Figure 1-13 A photograph of Oriental Frill type Blondiette from Naether's book, "The Book of Pigeon". (Naether, 1939).

Figure 1-14 An example of the Classical Oriental Frill pigeons. (Chai, 2014).

Figure 1-15 Modern Oriental Frill having shorter beak than the classical one (Gardner, 2015).

Not only in these two books, also in Lewis Wright's Practical Pigeon Keeper and Alice MacLeod's Pigeon Raising, this breed has been associated with Turkey (Wright, 1880) (MacLeod, 1913).

1.9 Molecular Marker Studies

1.9.1 The Mitochondrial DNA (mtDNA) of *Columba livia*

The entire mitochondrial genome of the rock pigeon (*Columba livia*) was characterized by Kan et al. (2010). Mitochondrial genome of *Columba livia* is shown in Figure 1-16. This molecule, about 17,229 base pairs long, contains 13 protein-encoding genes, 2 ribosomal RNA genes and 22 transfer RNA genes. In addition, it has a control region similar to that of other bird species. As with other vertebrates, most of these genes are encoded in the heavy strand (Kan et al., 2010). Complete mitochondrial genomes of feral pigeon, Ice pigeon (breed), Jacobin pigeon (breed) and Fancy pigeon (breed) are sequenced by Li et al, Zhang & He, He & Jia and Zhang et al., respectively (Li, Liu, & Wang, 2014) (Zhang & He, 2015) (He & Jia, 2015) (Zhang et al., 2015). The latest whole-genome assembly and gene annotation dataset for the rock pigeon are provided by Holt et al (2018).

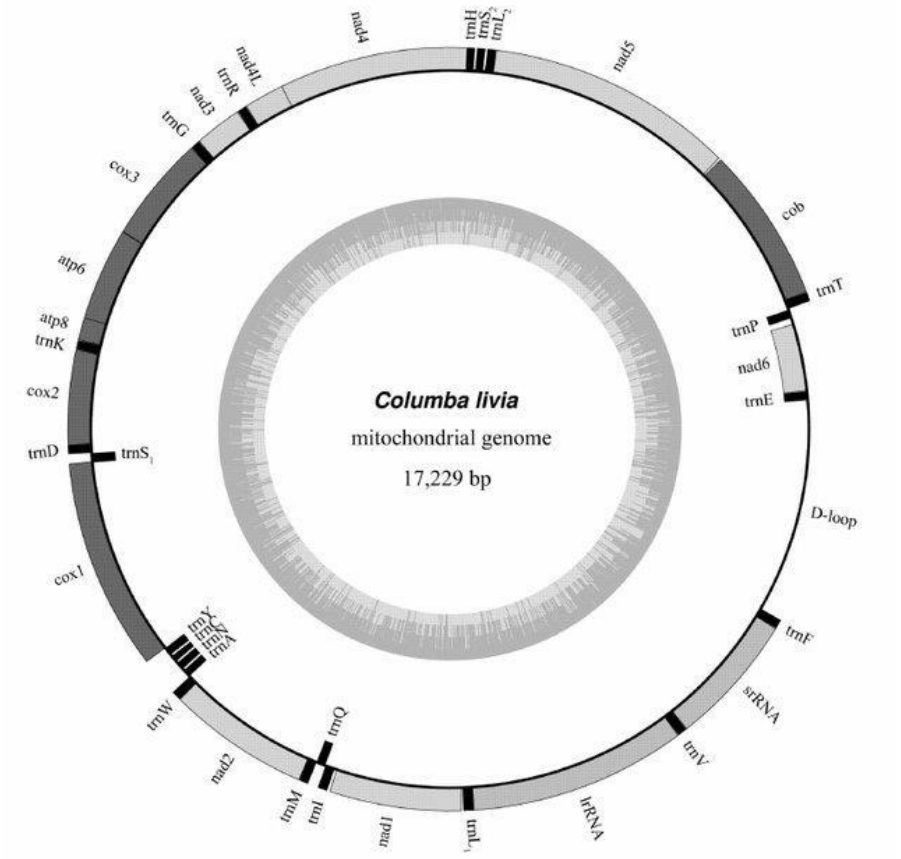

Figure 1-16 Columba livia mitochondrial genome. (Kan et al., 2010).

1.9.1.1 mtDNA *COI* and D loop markers

There are two mtDNA markers, *cytochrome c oxidase* (*COI*) and D loop, commonly used for barcoding and phylogenetic studies in birds. Hebert et al. (2003) proposed that genes can be used to distinguish species from one another and put forward the idea of "barcoding" living things. They proved their idea by showing that when *COI* used as barcode, it can place animals that have been sampled from high taxonomic levels sparsely, into the correct taxonomical group. Thus, using comparative *COI* data species-based distinction can be possible. Barcoding has been developed to be an easy, reliable and inexpensive species identification tool (Hebert, Cywinska, Ball, & DeWaard, 2003). Today, DNA barcoding is frequently used in areas such as

systematic, species identification, evolutionary biology, conservation research and ecology.

Hebert et al. (2004) analyzed 260 bird species in North America to test whether the *COI* gene could be used as an effective barcode for identifying different species of birds. Results demonstrated that the *COI* gene could be used as an effective barcode for bird species identification. This is an important study that has led to the rapid identification of new animal species using the *COI* barcode (Hebert, Stoeckle, Zemlak, & Francis, 2004).

For the *Columbidae* family, to resolve phylogenetic relationship among genera of *Columbidae*, the high barcoding capacity of the *COI* gene were utilized in the study of Khan & Arif (2013). A successful and reliable grouping has been established by using the *COI* gene at both genus and species level. The results of the study, thus showed that the *COI* gene region can be used as a reliable genetic barcoding marker. However, Ramadan et al (2011) conducted a study with a sample consisting of six different native Egyptian breed and one Japanese racing breed by using *COI* region and 11 microsatellite regions as markers. Results of the *COI* marker was only able to confirm that these breeds belonged to the same species. The statistical results of microsatellite loci have shown that the seven breeds studied were highly differentiated from each other. Thus, the results of the both markers should be evaluated carefully together when establishing sustainable conservation programs for commercially important breeds (Ramadan et al., 2011).

Since the D loop is more variable than other genes in mtDNA (Brown, George Jr., & Allan C. Willson, 1979) and only noncoding part of mtDNA, genetic diversity of avian D loop has been analyzed in many studies such as in genetic diversity and systematic evolution of China domestic ducks (H. F. Li et al., 2010) haplotype variation for the chicken (Guan, Geng, Silva, & Smith, 2007), the origins of Japanese native chicken (Oka et al., 2007) and a geographic and population study of the song sparrow (Fry & Zink, 1998). Due to this potential diversity of the D loop, it was also used in genetic studies related to the genus *Columba*. The analysis of partial region

of the D loop of American plain pigeon (*Columba inornata*) showed genetic conservation (Young & Allard, 1997). Similarly, species of *Columba bollii* and *Columba junonia* had only a few variable sites in their D loop (Marrero, Cabrera, Padilla, & Nogales, 2008). In contrast, D loop showed a high variability in other *Columba* species such as *Columba janthina janthina* and *Columba palumbus* (Seki, 2006) (Butkauskas, Švažas, Sruoga, Bea, & Grishanov, 2008). The analysis of complete D loop structure of pigeon (*C. livia*) showed variations of SNPs, a variable number of tandem repeats (VNTR) and STR (Tsai et al., 2009). In this study, the analysis of 131 samples of *C. livia* showed that the number VNTRs at the D loop region changed from 2 to 8 and reveals heteroplasmy. Also, the STR locus had multiple repeats within each individual. These findings indicate that polymorphic part of D loop can be useful markers to identify maternal relationships (Lee, Tsai, Liao, Linacre, & Hsieh, 2010).

1.10 Aim of the Study

Pigeons are accepted as a model organism since they can be easily bred and studied in the laboratory. Darwin has understood how selection generally works by help of them (Darwin, 1859). Domestic pigeons were used as a food source and ritual animals in history. Today there are about 350 distinct breeds of domestic pigeons and thousands of breeders. Breeds are selected for their different characteristics. The strong artificial selection applied on them has led to a strong directional selection of pigeon breeds, which results in extreme phenotypic diversity among the breeds. While many local breeds and others, different in behavior and morphology, have been present for a long time in Turkey, there is no any genetic and phylogenetic studies with regard to these breeds. Thus, the present study aims to complete the following studies using two different mtDNA markers, *COI* and D loop:

1. Design of specific primers for mitochondrial *COI* gene and the D loop region,

2. DNA sequence analysis of mitochondrial *COI* gene and the D loop region of local breeds in Turkey and breeds that are naive and originated from abroad and other breeds,

3. Determination of haplotypes for each mtDNA marker after editing and correcting the results of mtDNA sequences,

4. Determination of the extent of overlap between two markers by comparison with genetic results obtained from them,

5. Determination of phylogenetic relationships between local breeds and others and the level of genetic diversity within and between them by performing phylogenetic analysis,

6. Finally, to determine to what extent the genetic data overlaps in identifying breeds based on morphological characteristics. Thus, with the help of this study, it will be provided that pigeon breeding in Turkey to be supported by scientific data and done more consciously. It is also aimed to make important contributions to the development of strategies for the conservation of native breeds.

CHAPTER 2

MATERIALS AND METHODS

2.1 Sample Collection

The samples used in the study were obtained from local pigeon breeders. Breeders put them in the paper envelopes to convey them to our laboratory. Breeders have been informed for details about the collection and transmission of feather samples, such as not touching the root of the feathers with naked hands, not mixing feather samples taken from different individuals, and placing feathers in a paper envelope. The reliability of this sampling process was ensured by being in active communication with the breeders during the sampling period.

Samples were provided from Denizli, İzmir, Çanakkale, Bursa, Adana, Malatya, Konya, Uşak, Mersin and Australia. Most of the breeds used in the sampling are local, but the origin of 7 samples having the sample numbers 49 to 55 are entirely foreign. A table of all samples can be found in Appendix A.

Manisa Hünkarisi (Oriental Frill Pigeon) which is thought to been developed firstly in Turkey and the other short-beaked breeds which probably have origins in Middle East are represented with more samples than other breeds.

2.2 Genomic DNA Isolation, PCR and Sequencing

2.2.1 Genomic DNA Extraction from Feathers

In the structures like hair, horn and bird feather, cells begin to fill with keratin after cell formation and differentiation, known as keratinization. Keratinization makes it

difficult to obtain DNA from these structures. Thus, these tissues are required to be treated differently than other tissues during DNA isolation. Nevertheless, in many studies, DNA was obtained and amplified successfully from bird feather samples.

There are different views on exactly which parts of bird feathers are the source of DNA. According to Taberlet and Bouvet, 1991, pulp cells found at the far end of the feather (referred to as basal type or root) are the source of this DNA (Taberlet & Bouvet, 1991). However, the fact that DNA also can be obtained from the feathers of the museum samples shows that this claim may not be true. Some of these specimens have been preserved for more than 100 years. This has led to the idea that DNA can be obtained from the keratinized parts, too. Subsequent studies have supported this idea. However, the amount of keratinization may vary considerably depending on the species or individual factors such as age or metabolism of the individual. As harder feathers will be more keratinized, it may be more difficult to obtain DNA from them (Olsen, Bengtsson, Bertelsen, Willerslev, & Gilbert, 2012).

In the studies using bird feathers as a source of DNA, high yield and reproducible DNA was obtained from the part called calamus. However, there are studies showing that DNA can be obtained from barb and shaft parts (Olsen et al., 2012). When the feather growth is complete, the blood flow to the feather stops, but some of the blood cells remain in the so-called superior umbilicus (De Volo, Reynolds, Doglas, & Amtolin, 2008). In addition to calamus, Horvath et al. 2005 study shows that the blood clot found in this part of the feather is a valuable source of DNA (Horváth, Martínez-Cruz, Negro, Kalmár, & Godoy, 2005). Speller et al. (2011) also have successfully isolated and amplified DNA from the barb of feathers.

In the study of Horvath et al. 2005, the calamus part of the feathers was used as DNA source. However, if there is a blood clot in the super umbilicus, this part was also included in the isolation. Moreover, when the amount of DNA was insufficient, shaft part was also used. Different parts of feather are shown in Figure 2-1, Figure 2-2 and Figure 2-3.

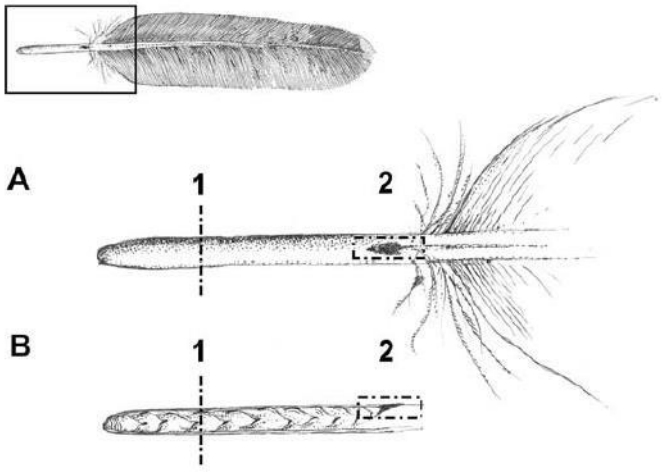

Figure 2-1 A. Posterior view of the root of the feather. B. Longitudinal cross section of the root of the feather. 1. Basal tip of the feather. 2. Superior umbilicus and blood clot it contains. (Horváth et al., 2005)

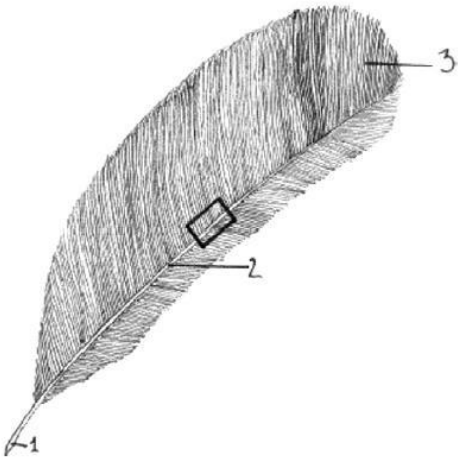

Figure 2-2 Pennaceous contour feather. 1. Calamus. 2. Shaft 3. Barb. (Olsen et al., 2012)

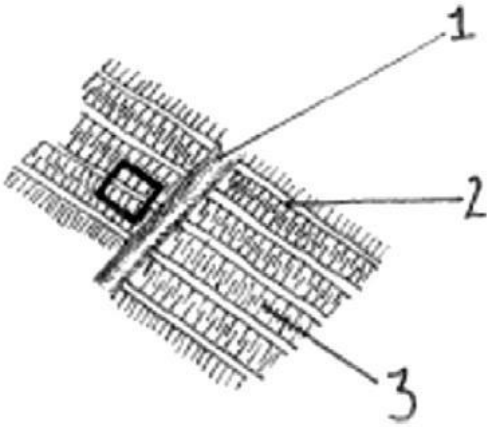

Figure 2-3 Detailed view of rachis, ramus and barbules. 1. Rachis 2. Ramus. 3. Distal and proximal barbules. (Olsen et al., 2012)

The feathers are stored in paper envelopes at +4°C until use (Taberlet & Bouvet, 1991) (De Volo et al., 2008). Before DNA isolation the feathers used in isolation were kept in distilled water for 15 min for cleaning. The calamus parts of the feathers were then cut into as small pieces as possible using sterile scalpel and forceps, first vertically and then horizontally. The cut pieces were put into 2 ml eppendorf tubes. If the feathers used are large or there is a large number of feathers for that sample, the cut pieces are placed in two different tubes in equal amounts. DNA isolation was performed by spin column method using Qiagen DNeasy Blood and Tissue Kit. In addition to the protocol provided by the company, DTT was added to the mixture of Animal Tissue Lysis buffer and Proteinase K enzyme used in the first step of isolation to break down the keratinized structures. DTT is prepared freshly every time since it is not stable after adding to the solution (Campos & Gilbert, 2012). The resulting DNA was dissolved in AE buffer to a final volume of 40 µl. The purity and concentration of DNA was determined at 260 and 280 nm using BioDrop µlite + spectrophotometer. The dissolved DNA was stored at -20 ° C for longer storage.

2.2.1.1 Steps of DNA Extraction from Feathers

1. 180 µl Buffer ATL, 20 µl proteinase K, and 20 µl 0,15 M DTT were added to a 1.5 ml microcentrifuge tube including tissue samples. DTT stock solution must be stored at –20°C. After adding all solutions tubes were vortexed for approximately 10 seconds.
2. Tubes were incubated at 56°C overnight and vortexed occasionally.
3. Tubes vortexed for approximately 10 seconds. 200 µl Buffer AL was added to the lysed samples and tubes vortexed briefly.
4. 200 µl ethanol (%96) was added to the mixture, vortexed briefly.
5. Mixtures including DNA were transferred to the spin columns provided by Qiagen. After transfer, samples were centrifuged for 1 minute at 8000 rpm. After centrifugation, collection tubes including supernatants were discarded.
6. Spin columns were placed into the new collection tubes. 500 µl AW1 solution was added to the spin columns. Samples centrifuged for 1 minute at 8000 rpm. After centrifugation, collection tubes including supernatants were discarded.
7. Spin columns were placed into the new collection tubes. 500 µl AW2 solution was added to the spin columns. Samples centrifuged for 3 minutes at 13000 rpm. After centrifugation, collection tubes including supernatants were discarded.
8. Spin columns were placed into the new collection tubes. Samples centrifuged for 1 minute at 13000 rpm to be sure that no ethanol remained in the silica membrane. After centrifugation, collection tubes including supernatants were discarded.
9. Spin columns placed in sterile 1.5 ml microcentrifuge tubes. 40 µl AE was added directly to the silica membrane. Samples centrifuged for 1 minutes at 10000 rpm.

2.2.2 Primer Design

As genetic markers, *cytochrome c oxidase subunit I* (*COI*) gene and D loop hypervariable region were selected to use. The primers required for amplification of these regions were designed based on the *Columba* livia mitochondrial genome (NC_013978.1) using the Primer3 web version 4.1.0 (Untergasser et al., 2012). Primers were commercially provided from Metabion company (Planegg, Steinkirchen, Germany). COXF1 – COXR1 and DLF2 – DLR2 primer pairs were used to amplify for *COI* gene region and D loop respectively. The sequence of primer pairs and their expected product lengths are shown in Table 2-1.

Table 2-1 Primer sequences and expected product lengths

Primer Name	Nucleotide Sequence	Product Length
COXF1	5’ – CGACTACAGCCTAACGCTTCA – 3’	810 bp
COXR1	5’ – GGAGATAATCCCGAATCCTG – 3’	810 bp
DLF2	5'- CTTGGCGCCTTTGGTTTT - 3'	850 bp
DLR2	5'- TTTCGGCGGTTACTTGTACG -3'	850 bp

2.2.3 DNA Amplification and Sequencing

PCR of both mtDNA genes was performed by preparing a reaction mix of 25 µl. including 1 µl of genomic DNA, 0.3 µl of each primer, 1x master mix (Solis BioDyne, 5x HOT FIREPol Blend PCR Mix). The reaction conditions of the primer pairs are as follows:

1. Reaction conditions for COX F1R1 primer pair:
    - 12 minutes/94 °C
    - 38 cycles consisting of 30 seconds/94 °C, 30 seconds/61 °C, 1 minute/72 °C

- 10 minutes/72 °C

2. Reaction conditions for DL F2R2 primer pair:
    - 12 minutes/94 °C
    - 40 cycles consisting of 30 seconds/94 °C, 30 seconds/61 °C, 1 minute/72 °C
    - 10 minutes/72 °C

In order to control contamination, negative controls were included within each PCR sets. All PCR products were separated on 1.5% of agarose gel for 75 minutes at 90 volts in standard TBE Buffer. The lengths of PCR products were determined using GeneRuler™ 100bp Plus DNA Ladder by screening on the Vilber Lourmat UV transilluminator.

After PCR purification, all products were sequenced in both forward and reverse direction to enhance accuracy of bases. Since PCR of D loop region resulted in more than one bands due to heteroplasmy within individuals, these bands were carefully cut out and extracted from the gel individually before sequencing reaction. All sequencing reactions were performed by using ABI terminator 3.1. Kit (Applied Biosystems Inc., Foster City, CA, USA). Electrophoresis and detection of fluorescently labeled nucleotides were conducted with an automatic DNA sequencer (ABI 3730x1 Genetic Analyzer, Applied Biosystems). Mitochondrial DNA sequences of both markers representing distinct haplotypes were recorded in order to be deposited in GenBank.

2.3 Data Analysis

2.3.1 Sequence Alignment

The alignment of both mtDNA *COI* and D loop gene sequences belonging to the 115 and 96 individuals respectively was conducted using the Clustal W algorithm (Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997) in MEGA X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). Alignments are checked by eye to provide

accuracy of alignments and corrected manually. Different haplotypes were determined in the program Arlequin 3.5.2.2 (Excoffier & Lischer, 2010). Mitochondrial DNA sequences both markers representing distinct haplotypes were recorded in order to be deposited in GenBank.

2.3.2 Genetic Diversity

Haplotype diversity (Hd) which is defined as "the probability that two randomly sampled alleles are different" (de Jong, Wahlberg, van Eijk, Brakefield, & Zwaan, 2011) and nucleotide diversity (π) which is defined as "the average number of nucleotide differences per site in pairwise comparisons among DNA sequences" (de Jong et al., 2011) (Nei, 1987) were calculated for both marker sets using DnaSP program v.6 (Rozas et al., 2017).

2.3.3 Haplotype Accumulation Curve

Haplotype accumulation curves are used to determine whether intra-specific sampling has been done adequately or not. These curves operate in the same logic as the rarefaction curves used in species richness determination (Gotelli & Colwell, 2001). The haplotype accumulation curves are plotted as a function of the number of samples and the cumulative mean number of haplotypes, and are evaluated according to whether they approach the asymptote at some point. In the beginning, as the number of samples is low, the curve rises very quickly, as a new haplotype will continue to be found in each sample. However, as the haplotypes found as the number of samples increases, the curve is expected to reach the saturation point. If the curve quickly reaches saturation, this indicates that much of the current haplotype diversity has been revealed. But, if the curve does not reach or is not tend to reach an asymptote, this means that sufficient sampling has not been done (Phillips, Gillis, & Hanner, 2019).

Haplotype Accumulation Curve calculated by using R statistical program. HaploAccum function belonging to the SPIDER package (Brown et al., 2012) was used to calculate Haplotype Accumulation Curves for both *COI* and D loop regions. Random permutation subsampling was done without replacement. A dataset including sequences from Turkey and no outgroups and no GenBank sequences were used to run the analysis. Slopes of the curves calculated by using the 10 last points of the curves (Phillips et al., 2019).

2.3.4 Phylogenetic Analyses

To determine phylogenetic relationships among Turkish local breeds and foreign breeds, phylogenetic analysis was performed separately for each marker. For mtDNA *COI* marker, 13 mtDNA *COI* sequences were downloaded from GeneBank and combined with *COI* haplotypes in this study (Table 2-2), and *Columba rupestris*, *Columba oenas* and *Columba palumbus palumbus* were used as outgroup (Table 2-3). Similarly, for mtDNA D loop marker 19 mtDNA D loop sequences (Table 2-4) were downloaded from GeneBank and combined with D loop haplotypes revealed in this study, and *Columba rupestris*, was used as outgroup (Table 2-5).

Table 2-2 Accession numbers of sequences used in the *COI* sequence alignment.

Accession Number	Species	Breed	Reference
AY666494.1	*Columba livia*	-	(Hebert, Stoeckle, Zemlak, & Francis, 2004)

Table 2-2 (continued)

FJ027421.1	*Columba livia*	-	(Kerr, Lijtmaer, Barreira, Hebert, & Tubaro, 2009)
GQ481605.1	*Columba livia*	-	(Kerr, Birks, et al., 2009)
GQ481606.1	*Columba livia*	-	(Kerr, Birks, et al., 2009)
GU571343.1	*Columba livia*	-	(Johnsen et al., 2010)
JF498842.1	*Columba livia*	-	(Kerr & Dove, 2011)
KF926376.1	*Columba livia*	Egyptian Swift	(C. H. Li, Shi, & Shi, 2015)
KP168712.1	*Columba livia*	Fancy Pigeon	(Zhang, Xu, et al., 2015)
KP258178.1	*Columba livia*	King Pigeon	(Zhang, He, & Xu, 2015)
KP306517.1	*Columba livia*	Ice Pigeon	(Zhang & He, 2015)

Table 2-2 (continued)

NC_013978.1	*Columba livia*	-	(Kan et al., 2010)
KJ722068.1	*Columba livia*	Archangel Pigeon	(Wu et al., 2016)
KF907308.1	*Columba livia*	Feral Pigeon	(C.-H. Li, Liu, & Wang, 2014)

Table 2-3 Accession numbers of *COI* sequences used as outgroups.

Accession Number	Species	Reference
NC_031867	*Columba rupestris*	(Soares et al., 2016)
KM926619.1	*Columba janthina janthina*	(Jang, Ryu, Kang, & Hwang, 2016)
NC_031868	*Columba jouyi*	(Soares et al., 2016)

Table 2-4 Accession numbers of sequences used in the D Loop sequence alignment.

Accession Number	Species	Breed	Reference
KJ722068.1	*Columba livia*	Archangel Pigeon	(Wu et al., 2016)

Table 2-4 (continued)

KP306517.1	*Columba livia*	Ice Pigeon	(Zhang & He, 2015)
KP258178.1	*Columba livia*	King Pigeon	(Zhang, He, et al., 2015)
KP168712.1	*Columba livia*	Fancy Pigeon	(Zhang, Xu, et al., 2015)
FJ792689.1	*Columba livia*	-	(Tsai, Lee, Liao, Weng, et al., 2009)
FJ792693.1	*Columba livia*	-	(Tsai, Lee, Liao, Weng, et al., 2009)
FJ792695.1	*Columba livia*	-	(Tsai, Lee, Liao, Weng, et al., 2009)
FJ792690.1	*Columba livia*	-	(Tsai, Lee, Liao, Weng, et al., 2009)
FJ792697.1	*Columba livia*	-	(Tsai, Lee, Liao, Weng, et al., 2009)

Table 2-4 (continued)

FJ792691.1	*Columba livia*	-	(Tsai, Lee, Liao, Weng, et al., 2009)
FJ792692.1	*Columba livia*	-	(Tsai, Lee, Liao, Weng, et al., 2009)
FJ792698.1	*Columba livia*	-	(Tsai, Lee, Liao, Weng, et al., 2009)
FJ792694.1	*Columba livia*	-	(Tsai, Lee, Liao, Weng, et al., 2009)
FJ792696.1	*Columba livia*	-	(Tsai, Lee, Liao, Weng, et al., 2009)
KF926376.1	*Columba livia*	Egyptian Swift	(C. H. Li et al., 2015)
KF907308.1	*Columba livia*	-	(Zhang & He, 2015)
GQ240309.1	*Columba livia*	-	(Tsai, Lee, Liao, Linacre, & Hsieh, 2009)

Table 2-4 (continued)

KP319029.1	*Columba livia*	-	(He & Jia, 2014)
NC_013978.1	*Columba livia*	-	(Kan et al., 2010)

Table 2-5 Accession number of D Loop sequence used as outgroup

Accession Number	Species	Reference
NC_031867	*Columba rupestris*	(Soares et al., 2016)

2.3.4.1 Model of Sequence Evolution

Based on Bayesian Information Criterion (Schwarz, 1978), the best evolutionary sequence model for both data sets was chosen using MEGA X (Kumar et al., 2018). The best-fit molecular evolution model for mitochondrial *COI* gene (31 sequences) was the Hasegawa-Kishino-Yano (HKY) (Hasegawa, Kishino, & Yano, 1985) with gamma distributed site rate variation, using 4 discrete mutation classes (ln L= -1504.337, BIC= 3646.984) and the best-fit molecular evolution model for mitochondrial D loop (31 sequences) gene was the Hasegawa-Kishino-Yano (HKY) model (Hasegawa et al., 1985) (ln L=- -1679.61, BIC= 3718.92) with uniform site rate variation.

2.3.4.2 Test of Molecular Clock

The molecular clock test was performed by comparing the ML value for the given topologies of *COI* data set and D loop data set with and without the molecular clock constraints under HKY model (Hasegawa et al., 1985) with gamma distributed site

variation and uniform site variation respectively, using MEGA X (Kumar et al., 2018) software. The null hypothesis of equal evolutionary rate throughout the tree was rejected at a 5% significance level (P = 0.05) for both mitochondrial *COI* gene and D loop.

2.3.4.3 Construction of Phylogenetic Trees

Bayesian phylogenetic of both mitochondrial *COI* and D loop data sets were performed in the program BEAST version 1.10 (Suchard et al., 2018). The data sets were prepared using the BEAST assistance program BEAUTI 1.7.5. For both mitochondrial *COI* data set Bayesian analysis was initiated from the random starting tree the Hasegawa-Kishino-Yano (HKY) mutation model (Hasegawa et al., 1985) with gamma distributed site variation, using 4 discrete mutation classes. For D loop data set, Bayesian analysis was initiated from the random starting tree the Hasegawa-Kishino-Yano (HKY) mutation model (Hasegawa et al., 1985) using 4 discrete mutation classes. For both data sets, a relaxed molecular clock (Drummond, Ho, Phillips, & Rambaut, 2006) was estimated using an uncorrelated lognormal prior and the Coalescent: Expansion Growth Model was selected as tree prior for *COI* and Coalescent: Constant Size Model was selected as tree prior for D loop. The MCMC was run for 100,000,000 steps and sampled every 10000 steps to calculate posterior distribution parameters. The first recorded 1000 trees (10% of the sampled trees) were discarded as burn-in. The effective sample sizes for posterior probability were evaluated from the logfiles in TRACER 1.7 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018).

Phylogenetic trees were also constructed in the MEGA X (Kumar et al., 2018) with Maximum Likelihood method by using Hasegawa-Kishino-Yano model (Hasegawa et al., 1985) with gamma distributed site rate variation (HKY+G) for *COI* and HKY model for D loop. The validity of the tree was assessed using bootstrapping (Felsenstein,1985) with 1000 replications.

2.3.5 Median Joining Network

A median-joining network was constructed with the program NETWORK version 4.6.1.1 (Bandelt et al., 1999) to reveal evolutionary relationships based on mutational variations and probable ancestral connections among different haplotypes. In the construction of network, 28 *COI* haplotypes were used. Outgroup sequences were removed from the network analysis. Since D loop sequences have VNTRs with distinct numbers at their ends, the network is not constructed for D loop sequences. Clusters are colored according to the grouping have been made based on both the ELFP (European Association of Poultry, Pigeon- and Rabbit breeders) (European Association of Poultry Pigeon and Rabbit breeders, 2018) grouping and information provided by the Turkish pigeon breeder, Murat Türkeş.

2.3.6 Demographic Analyses

To test the possible population expansion occurred, several statistical tests used. Confidence intervals of those tests were calculated by using Coalescent Simulations provided by the program DnaSP v.6 (Rozas et al., 2017). Simulation was run under a Growth Model. Simulation was repeated 10.000 times.

2.3.6.1 Mismatch Distribution Analysis

While mismatch analysis is based on pairwise differences between all sequences, mismatch distribution graphs show the distribution of these differences between sequences. Certain patterns in the mismatch distribution graphs are thought to give clues about population expansion (Venkatesan, Westbrook, Hauer, & Rasgon, 2007). The demographic history of populations determines the shape of the mismatch distribution. The unimodal mismatch distribution typically indicates population expansion, whereas the ragged and/or multimodal mismatch distribution indicates demographic equilibrium or long-standing constant populations (Rogers &

Harpending, 1992). DnaSP is able to calculate both observed and expected mismatch distributions in stable populations as well as in growing/declining populations. Also, it calculates the raggedness statistics which quantifies the smoothness of mismatch distribution (Venkatesan et al., 2007).

Mismatch distribution analysis was done in DnaSP v.6 (Rozas et al., 2017) software by using constant population size model and population growth/decline model respectively. Outgroups and GenBank sequences were not included to the analysis.

2.3.6.2 Fu an Li's Test

based on mutations in both older and younger branches (Venkatesan et al., 2007). While negative values of Fu and Li's D* and F* indicate selective-sweep, recent population growth, or background selection, it is known that these statistics are more sensitive to background selection (Venkatesan et al., 2007) (Lopes, Miño, & Del Lama, 2007). Fu and Li's Test (Y. X. Fu & Li, 1993) has been done in DnaSP v.6 (Rozas et al., 2017) software. Outgroups and GenBank sequences were not included to the analysis.

2.3.6.3 Tajima's D

Tajima's D detects the shifts from neutrality either because of population expansion or selection (Tajima, 1989) (de Jong et al., 2011). Tajima's D Test has been done in DnaSP v.6 (Rozas et al., 2017) software. Outgroups and GenBank sequences were not included to the analysis.

2.3.6.4 Fu's Fs Test

Fu's Fs Test uses the distribution of alleles or haplotypes to show the effect of the sudden population expansion or genetic hitchhiking on the allele frequency (Yun Xin Fu, 1997) (de Jong et al., 2011). The allele frequency spectrum shifts from the neutral

Wright-Fisher Model when a non-neutral process occurs (de Jong et al., 2011). Fu's Fs is thought to be the most powerful test to detect population growth (Yun Xin Fu, 1997).

Fu's Fs Test (Yun Xin Fu, 1997) has been done in DnaSP v.6 (Rozas et al., 2017) software. Outgroups and GenBank sequences were not included to the analysis.

2.3.6.5 Strobeck's Statistics

Strobeck's Statistics was calculated in DnaSP program v.6 to infer the probability of having an equal number or fewer haplotypes than observed (h) (Strobeck, 1987). Outgroups and GenBank sequences were not included to the analysis

CHAPTER 3

RESULTS

3.1 Mitochondrial DNA Isolation from Feathers

Mitochondrial DNA isolation of all samples performed using QIAGEN DNeasy Blood and Tissue Kit was successful. Concentration of isolated genomic DNA was ranging from 0 to 149 ng/µ.

3.2 PCR

While all of the 115 individuals were successfully amplified for *COI* gene region, 96 of them were able to be amplified for D loop. Examples of PCR results for *COI* and D loop can be seen below in Figure 3-1 and Figure 3-2, respectively. The PCR products of *COI* gene region is about 780 bp in length. Agarose gel images of the PCR products of the D loop region indicate that some individuals of *Columba livia* carry multiple bands with different lengths of mitochondrial D loop. Lengths of the PCR products of D loop range between 835 and 1019 bp.

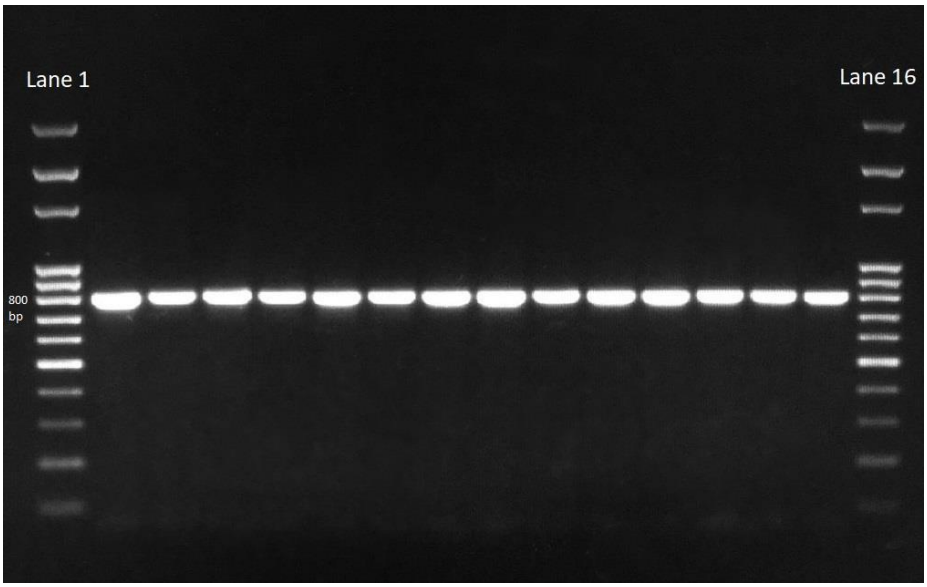

Figure 3-1 Amplified mitochondrial *COI* gene. Lane 1 and lane 16 are the DNA ladders (Solis BioDyne 100 bp DNA Ladder Ready to Load). Lane 2-15 are 780 bp PCR products of *COI* gene.

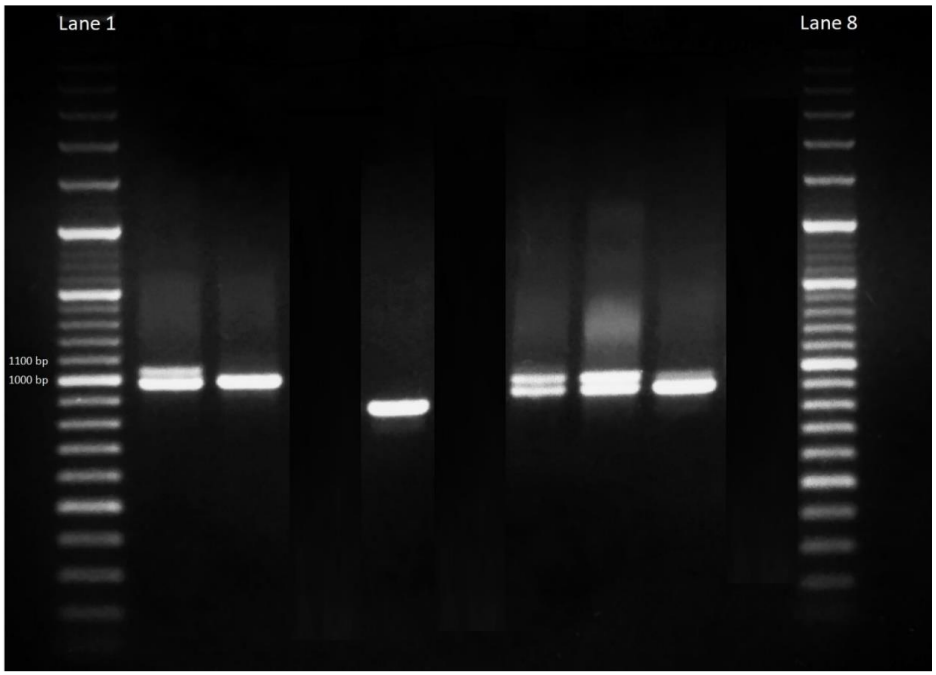

Figure 3-2 Amplified mitochondrial D loop region. Lane 1 and lane 8 are the DNA ladders (Thermo Scientific O'RangeRuler 100 bp+500 bp DNA Ladder, ready-to-use). Lane 2-7 are the PCR products of D loop and their lengths range from 835 to 1019 bp. It can be seen that 2, 5 and 6 carry multiple bands, indicating the heteroplasmy.

3.3 Results of Data Analyses

3.3.1 Genetic Diversity of Mitochondrial *COI* Gene

Values of haplotype (h) and nucleotide diversity (π) (Nei, 1987) calculated for *COI* gene using DnaSP (Rozas et al., 2017) program were $\pi = 0.00151$ and $h = 0.661$, respectively. According to these results, despite low nucleotide diversity, haplotype diversity seems at moderate level.

3.3.2 Mitochondrial *COI* Haplotypes

115 mitochondrial *COI* sequences were analyzed for this study. Average nucleotide compositions calculated for all individuals were 26.36 % for T, 33.04 % for C, 24.26 % for A and 16.34 % for G. For all individuals, 9 parsimony informative sites (1,216 %), 13 variable sites (1.756 %) and 4 singleton sites (0.54 %) were found. According to the analysis done by the program Arlequin 3.5.2.2 (Excoffier & Lischer, 2010), 21 distinct haplotypes were detected. Unique haplotypes and SNPs are shown in Table 3-1. Haplotype 1 (Hap_1) is the most frequent haplotype.

126 sequences were analyzed together with the GenBank sequences. Those sequences were belonged to 45 different breeds. Most frequent *COI* haplotype, Hap 1 was carried by 68 individuals belonging to 37 different breeds. Hap 7 was only carried by Modern Oriental Frill breed.

Table 3-1 Haplotypes of *C. livia* breeds used in this study detected by sequencing mitochondrial *COI*

Positions	35	53	113	121	136	170	389	461	521	551	680	694	707	710	713	725	727	732
Haplotypes																		
Hap_1	C	C	G	C	G	C	T	T	A	T	A	T	T	T	T	C	T	T
Hap_2														C				
Hap_3																A		
Hap_4										C								
Hap_5												C					A	
Hap_6			A															
Hap_7													C					
Hap_8															G	A		
Hap_9														C		A		
Hap_10														C		A		
Hap_11															G	A		
Hap_12																A		
Hap_13					A					C								
Hap_14								C										
Hap_15			C						G			C						
Hap_16												C						
Hap_17										C		C					A	
Hap_18														C			A	
Hap_19												C		C			A	
Hap_20																		G
Hap_21																		
Hap_22						T					G							
Hap_23						T												
Hap_24				G		T												
Hap_25	T																	
Hap_26							C							C				
Hap_27			C						G									
Hap_28		T																

3.3.3 Haplotype Accumulation Curve of Mitochondrial *COI* Gene

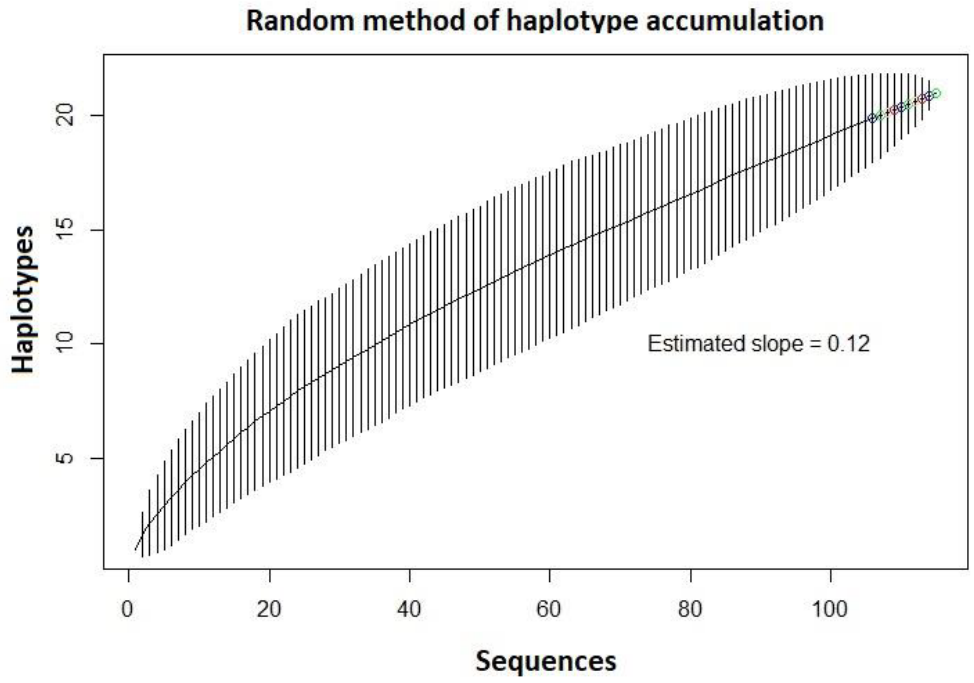

Figure 3-3 Haplotype Accumulation Curve of *COI* region. Y-axis shows number of haplotypes that is identified while the number of sampled sequences (X-axis) increases. Colored dots at the end of the curve show the last 10 points which were used to estimate.

The slope of the Haplotype Accumulation Curve plotted for the *COI* gene region was calculated as 0.123 using the last ten points in the curve. This number means that an average of 1.23 new haplotypes can be identified in every 10 new samples. The curve does not tend to reach an asymptote. A clear threshold value was not accepted in this study to determine the satisfaction of the curve. And the threshold values determined in other studies are generally arbitrary (Phillips, Gillis, & Hanner, 2019). In addition, this number (0.123) is so high that there is no doubt that sampling is currently insufficient. That is, adding more samples to the study has potential to change significantly the results here. It is known that sampling, especially the studies in which mitochondrial DNA is used as marker to reveal genetic diversity, has a tremendous effect on the results of the study. Genetic diversity is affected from

migration/gene flow, mutation, natural selection and random genetic drift. For example, impact of genetic drift strongly depends to the population size and is higher when population is small (Phillips et al., 2019). Thus, genetic diversity studies based on this mitochondrial marker should include as many samples as possible to eliminate biased estimate of genetic diversity and other types of analysis.

3.3.4 Genetic Diversity of Mitochondrial D loop

Values of haplotype (h) and nucleotide diversity (π) (Nei, 1987) which were calculated for D loop using DnaSP (Rozas et al., 2017) program were $\pi = 0.00020$ and $h = 0.882$ respectively. Similar to mitochondrial *COI* gene, haplotype diversity is quite high even more than seen in *COI* gene due to heteroplasmy in the D loop haplotypes.

3.3.5 Mitochondrial D loop Haplotypes

96 mitochondrial D Loop sequences analyzed for this study. Average nucleotide compositions calculated for all sequences were 31.43 % for T, 28.78 % for C, 28.18 % for A and 11.6 % for G. For all individuals, 10 parsimony informative sites (0.973 %) and 19 variable sites (1.85 %) were found.

115 sequences were analyzed together with the GenBank sequences. Those sequences were belonged to the 45 different breeds. Most frequent D loop haplotype, Hap 13 carried by 39 individuals belonging to 21 different breeds. Second most frequent D loop haplotype was Hap 14, carried by 34 individuals belonging to the 26 different pigeon breeds. Haplotypes 21, 22, 23 and 26 were only carried by the Modern Oriental Frill individuals.

Table 3-2 Haplotypes of *C. livia* breeds used in this study detected by sequencing mitochondrial D loop.

Positions	83	145	187	275	276	391	447	459	460	503	514	540	547	568	580	602	619	685	700	737	746	798	868
Haplotypes																							
Hap_1	C	G	G	C	C	T	T	C	T	T	T	C	T	T	T	T	C	G	C	T	G	T	G
Hap_2						-																	
Hap_3																							
Hap_4						-																	
Hap_5		-																					
Hap_6		-				-																	
Hap_7						-						T											
Hap_8												T											
Hap_9	G						C			-			-			-							
Hap_10						-				-			-	-		-							
Hap_11				T	G	-		T	C	-	-		-	-		-							
Hap_12																	T		G				
Hap_13																							
Hap_14						-																	
Hap_15																			C			C	
Hap_16						-													C			C	
Hap_17																							
Hap_18						-																	
Hap_19		-																					
Hap_20		-				-																	
Hap_21															C			A			A		A
Hap_22		A													C			A			A		A
Hap_23		A													C			A			A		A
Hap_24																							
Hap_25						-																	
Hap_26						-									C			A			A		A
Hap_27			C																				
Hap_28			C			-																	
Hap_29																							
Hap_30						-																	

3.3.6 Heteroplasmy in Mitochondrial D loop

According to the sequencing results, a 61 base pair long VNTR (Variable Number Tandem Repeat) was seen at the end of D loop. VNTR regions in *C. livia* and *C. rupestris* used as outgroup was shown in Table 3-3. This VNTR region was previously detected by two different studies (Tsai et al., 2009) (Lee et al., 2010).

Table 3-3 61 bp long VNTR regions of *Columba livia* and *Columba rupestris*. The only different base was shown in red as bold and underlined.

Species	Sequence	Length
C. livia	TTGTCCAAAACATTA**G**ACCAATTTAAGCCACTCTCCTCATCACCCGCTCACTCACCAACTC	61 bp
C. rupestris (outgroup)	TTGTCCAAAACATTA**A**ACCAATTTAAGCCACTCTCCTCATCACCCGCTCACTCACCAACTC	61 bp

Also, the sequencing results showed that some of the individuals are heteroplasmic, meaning that they carry more than one type of mitochondrial DNA molecule for D loop (Huang et al., 2019) (Paneto et al., 2007). These different mitochondrial DNA molecules have different SNPs, deletions and different number of VNTRs. Haplotypes including different numbers of repeats showed in Table 3-4.

Table 3-4 VNTR repeat numbers of D loop haplotypes.

Haplotype	Number of repeats	Number of samples
Hap_1	2	3
Hap_2	2	3
Hap_3	3	31
Hap_4	3	31
Hap_5	3	2
Hap_6	3	2
Hap_7	3	1
Hap_8	3	1
Hap_9	3	1
Hap_10	3	2
Hap_11	3	1
Hap_12	3	1
Hap_13	4	39
Hap_14	4	34
Hap_15	4	1
Hap_16	4	1
Hap_17	5	17
Hap_18	5	14
Hap_19	5	1
Hap_20	5	1
Hap_21	5	1
Hap_22	5	1
Hap_23	5	1

Hap_24	6	12
Hap_25	6	3
Hap_26	6	1
Hap_27	6	1
Hap_28	6	1
Hap_29	7	4
Hap_30	7	3

Length heteroplasmy in pigeon mitochondrial D loop was primarily detected in the studies of (Tsai et al., 2009) and Lee et al., (2010). We also found a length heteroplasmy in the T stretch covering the region from 382nd position to 391st position of D loop sequences used in this study. Same individuals have both T stretches with 10 thymines and 9 thymines bases at position 382-391. According to alignment based on these information, 30 unique haplotypes defined using the program Arlequin 3.5.2.2 (Excoffier & Lischer, 2010). D loop structure of *Columba livia* defined by Tsai et al. was shown in Figure 3-4 (Tsai et al., 2009).

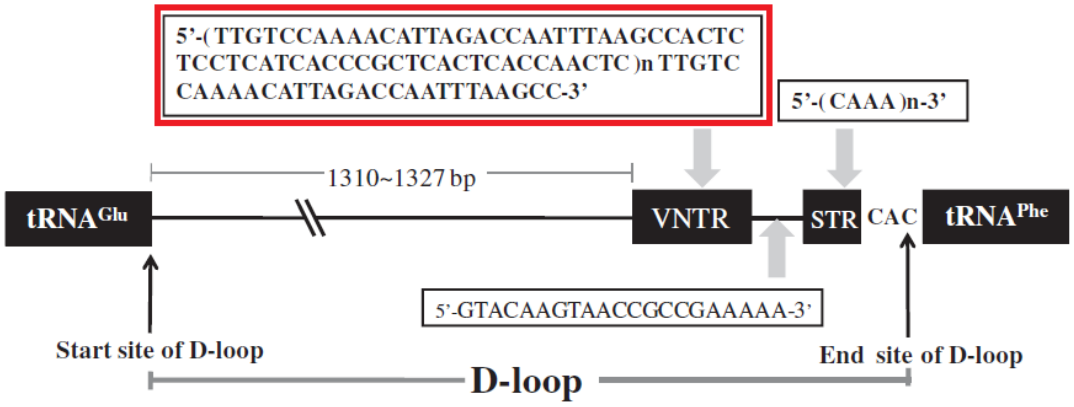

Figure 3-4 D loop structure of *Columba livia*, defined by Tsai et al. (Tsai et al., 2009). 61 bp VNTR region was emphasized in a red rectangle.

Heteroplasmies can be point-based or length-based heteroplasmies (Paneto et al., 2007). In the length heteroplasmy, there are at least two types of mitochondrial DNA molecules in a DNA extract that they have distinct number of nucleotides so different lengths (Berger et al. 2011). In the point heteroplasmy, an individual carries different

mtDNA haplotype which are different from each other at a single or few nucleotide position (Gandolfi et al., 2017). Heteroplasmy might be inherited maternally (Ladoukakis & Zouros, 2017), can be caused by somatic mutations (Latorre-Pellicer et al., 2019) or can be the result of numts (Gandolfi et al., 2017). Age of the sampled individual or hair/feather color can affect the presence of heteroplasmy (Spicer, Kun, Sacks, & Wictum, 2014). Tissue differences also have effects on the frequency of heteroplasmy (Huang et al., 2019) (Paneto et al., 2007). Errors in PCR and DNA extraction steps can cause heteroplasmy, too (Gandolfi et al., 2017) (Paneto et al., 2007).

Heteroplasmy is thought as a transient event in evolution, disappearing in several generations (Huang et al., 2019) (Breton & Stewart, 2015). Huang et al. (2019)'s results suggest a clear maternal inheritance of heteroplasmy, and its occurrence decreases a lot, even over single generation in chicken (Huang et al., 2019). They also showed that 81% of the heteroplasmies are tissue-specific and most of the heteroplasmies are caused by somatic mutations. This can explain the change of heteroplasmy abundance among generations. In this study, thus, it was thought that the detected heteroplasmies can be the results of somatic mutations. Therefore, D loop data might not provide a reliable phylogenetic result for the breeding structure of pigeon breeds (Huang et al., 2019). There are also examples of maternally inherited heteroplasmy such as the study of Doublet et al. (Doublet, Souty-Grosset, Bouchon, Cordaux, & Marcadé, 2008). They identified a heteroplasmic state in terrestrial isopod crustaceans which has conserved for over 30 million years possibly as it contains two mutational types of a single tRNA locus producing two different mitochondrial tRNAs (Doublet et al., 2008) (Doublet et al., 2012).

Numts are the parts of the mitochondrial DNA was detected in the nuclear genomes (Huang et al., 2019). They can cause contamination and consequently heteroplasmy. At least 13 numts were detected in the chicken genome by Pereira et al. (Pereira, Johnson, Clayton, & Baker, 2007). Lengths of the numts are usually shorter than the length of the amplified gene (Gandolfi et al., 2017). In our study, any short bands were not observed on the gel under the desired bands. Besides, heteroplasmic bands

were cut from the gel separately. We also used back to back primers to prevent genomic DNA contamination (Huang et al., 2019). Thus, it can be concluded that heteroplasmies in our study are not caused by numt contamination. Nested primers can also be used to exclude the possibility of numt contamination like Gandolfi et al. did (Gandolfi et al., 2017).

There are several evidences about the effect of the hair color on heteroplasmy in dog and human hairs (Calloway, Reynolds, Herrin, & Anderson, 2000) (Spicer et al., 2014). In the study of Spicer et al., two dog hairs including heteroplasmies were lighter in color and two of the total three heteroplasmies belonged to the sample taken from the oldest dog in the study (Spicer et al., 2014). It can indicate that frequency of heteroplasmy might be affected by the factor like age and hair color. De Camargo et al. (2011) however, could not find any relationship between age and heteroplasmy (De Camarago et al., 2011). In our study, no evaluation has been done about the relationship between heteroplasmy and feather color or the age of the bird.

In forensics, studies about heteroplasmy in the mtDNA are abundant. Paneto et al. (2007) compared the heteroplasmy cases coming from the blood and hair samples of same individual. They concluded that samples coming from different tissues, especially hair samples, may contain different amount of heteroplasmies and should be treated with caution (Huang et al., 2019) (Paneto et al., 2007). They found that hair shafts are more heteroplasmic than blood. Hair follicles are naturally clonal and their keratinization process requires high amount of energy. These factors may cause to the segregation of heteroplasmic variants in mtDNA from hair shafts. (Paneto et al., 2007). Paneto et al. (2007) used only one hair shaft for each individual to avoid the possibility of heteroplasmy that may result from different hair shafts. In this study, however, more than one feather was used for each pigeon since the amount of DNA obtained from a single feather was insufficient for genetic analysis. Thus, it is not possible to make a definitive conclusion in this study whether the heteroplasmies observed in individuals are caused by the use of different feathers of the individual or not.

Additionally, in several studies (Paneto et al., 2007) (Gandolfi et al., 2017), in order to ensure that the observed heteroplasmies were not caused of the errors in the PCR or DNA extraction steps, the DNAs obtained from old and new isolations were duplicated by double PCR reactions and the heteroplasmic pattern was tried to be confirmed. Guang-Xin et al. (2018) demonstrated that heteroplasmies can be caused by the unspecific replication during PCR amplification. In our study, it was not possible to double every PCR reaction or isolation due to absence of enough tissue resources.

3.3.7 Haplotype Accumulation Curve of D Loop Haplotypes

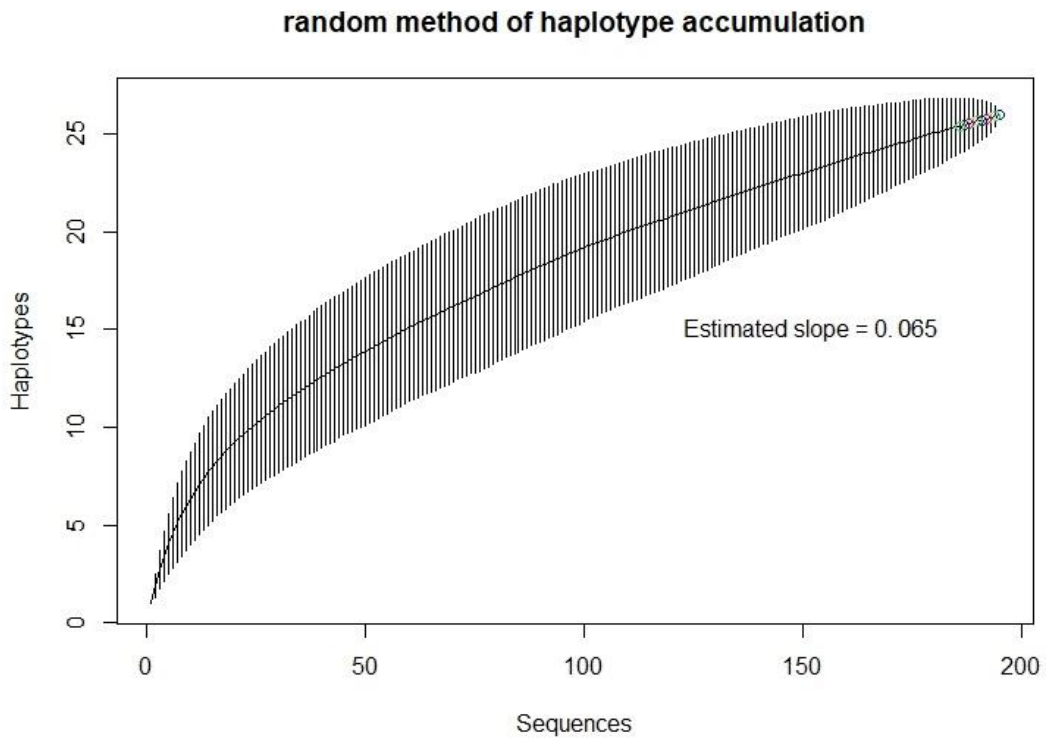

Figure 3-5 Haplotype Accumulation Curve of D loop region. Y-axis shows number of haplotypes identified while the number of sampled sequences (X-axis) increases. Colored dots at the end of the curve show the last 10 points which were used to estimate slope.

The same steps for the *COI* gene region were also applied for the D Loop dataset, and the slope of the drawn curve was calculated as an average of 0.065. This result means that 6.56 new haplotypes can be identified in every 100 samples. A clear threshold value was not accepted in this study to determine the satisfaction of the curve.

The general acceptance is that a slope of 0.01 and below strongly proves that sampling is sufficient, and that a slope of 0.1 and above strongly proves that sampling is inadequate (Phillips et al., 2019). However, in the D loop, the number of haplotypes is high due to heteroplasmy. This much of sampling may not be sufficient for samples without heteroplasmy. It should not be forgotten that the functional forms of such curves are not fully known and these forms may differ considerably between taxonomic units. In addition, the determination of the threshold slope value is quite arbitrary (Phillips et al., 2019).

3.4 Results of Phylogenetic Analysis

3.4.1 Results of Bayesian Phylogenetic Analysis of *Columba livia COI* Haplotypes

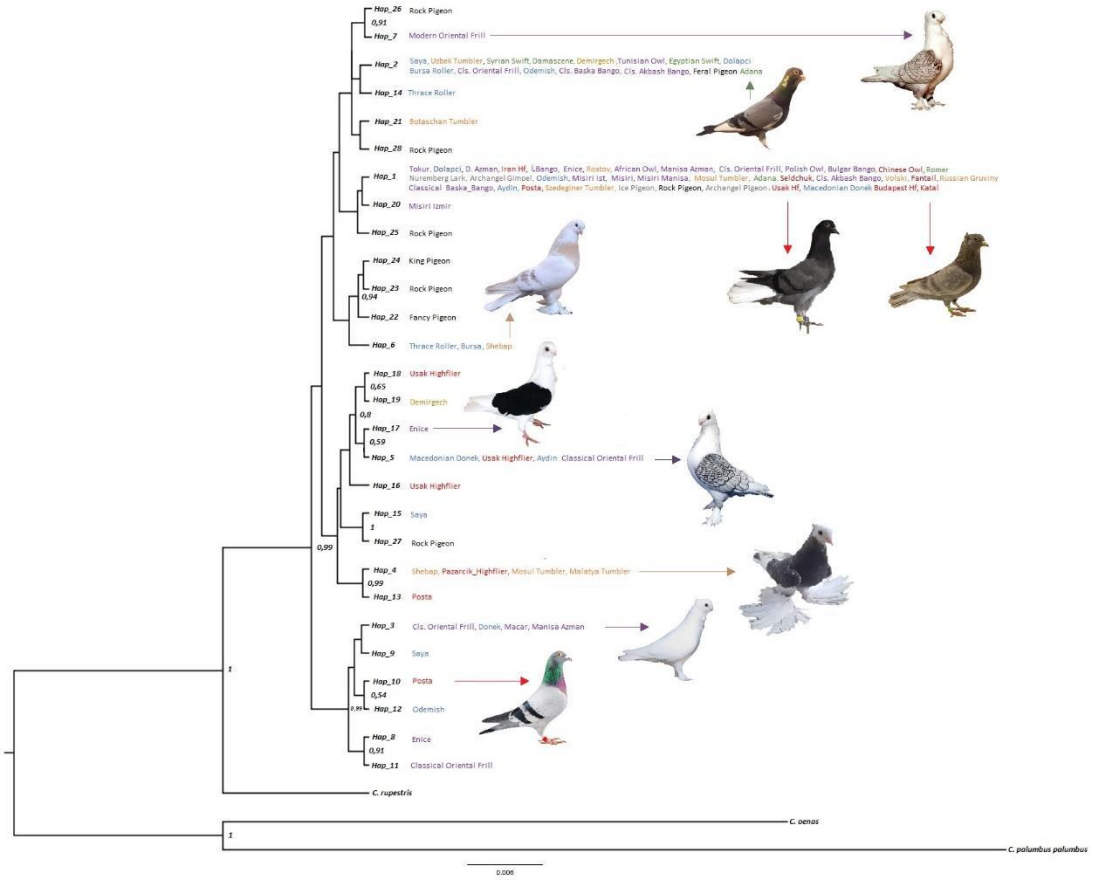

Figure 3-6 Bayesian Phylogenetic Tree of *COI* haplotypes. Numbers indicate the posterior probabilities of the branching structures. Colors indicate different pigeon groups based on the classification in the Table 3-5.

As the results of the Maximum Likelihood and Bayesian phylogenetic trees constructed in this study were consistent, only the result of the Bayesian analysis was discussed here (Figure 3-6). Results of the Maximum Likelihood phylogenetic trees are shown in Appendix. Posterior probabilities of the constructed branches are generally low. There is no clear divergence among distinct breeds. It means that individuals of distinct breeds do not form a separate clade in the result of

phylogenetic analysis. Even in some cases haplotypes of breeds can distribute into different clades. One of the possible reasons for that is the low number of informative sites found in the data. The other possible explanation can be the high level of introgression among distinct breeds caused by the selection that humans applied for particular traits such as color, pattern, or feathered feet by artificial selection. This might not hold true for some breeds, such as the Modern Oriental Frills. There were two individuals of the Modern Oriental Frill breed in this study and two of them have been constructed one distinct clade. Introgression might have transferred a particular haplotype of one breed to another. This could result in the construction of undifferentiated clades for breeds.

According to the Bayesian analysis of *Columba livia* haplotypes of Turkey; Haplotypes 27 and 15 constructed a clade with the posterior probability equals to 1. Haplotypes 4 and 13 have the posterior probability equals to 0.99.

Haplotype 27 includes only the one sequence with no breed information having the GenBank accession number FJ027421.1. This GenBank sample was collected from Argentina, Buenos Aires according to the BOLD (Barcode of Life Database) accession number (KBARG055-07) given in the associated paper (Kerr, Lijtmaer, Barreira, Hebert, & Tubaro, 2009). Haplotype 15 includes only one individual belonging to the breed “Saya”.

Haplotype 4 includes Shebap Tumbler, Pazarcik Highflier, Mosul Tumbler and Malatya Tumbler breeds. Tumbler pigeons are raised for their acrobatic skills. Haplotype 13 is carried by Posta Pigeon (Classical Homer Pigeon). As classical homer pigeons have been improved in Belgium and England with the contribution of lots of different breeds (Shapiro et al.), it has been found that classical homer pigeons clustered with different other pigeon breeds in the study of Shapiro et al. (Shapiro et al). This might seem unrelated at first glance but it has been stated that the reason for this grouping might be the contribution of these different breeds to the production of homer pigeons. A similar situation might be valid for our Posta pigeon too. But neither Posta pigeon nor the breeds grouped with it are not related to the

European breeds. Most of the breeds in question are probably belong to the Middle East. Also, other Posta pigeons in this study were grouped with different other breeds, too. Consequently, in the light of the available data, this explanation does not go beyond being a possibility.

Haplotypes 3, 8, 9, 10, 11 and 12 formed a clade having the posterior probability value equals to 0.99. Haplotype 3 included the breeds Classical Oriental Frill, Donek, Macar, Classical Oriental Frill and Manisa Azman; Haplotype 8 included the breed Enice; Haplotype 9 included the breed Saya; Haplotype 10 included the breed; Haplotype 11 included the breed Classical Oriental Frill and Haplotype 12 included the breed Odemish. These breeds are belonged to the Owl (Classical Oriental Frill and Enice), Spinner/Roller (Saya and Odemish) and Highflier (Posta) breed groups. There is no known connection between those breed groups. These samples provided from different cities and poultries.

Haplotype 8 and Haplotype 11, constructed a branch having the posterior equal to 0.91. Both of the individuals in these haplotypes are sampled from the poultry of Muzaffer Göl. This could be a one of the possible explanations for this clade. Also, both of these breeds (Enice and Classical Oriental Frill) are short beaked and small bodied, “owl type” breeds. Enice (or Yenice) can be classified as “Misirli” or “Misiri” according to the breeders and Misiri breeds are also classified as owl type. Oriental Frill pigeons are classified as owl type pigeon breed, too. (European Association of Poultry Pigeon and Rabbit breeders, 2018). According to the information obtained from both historical sources and breeders, these two breeds can be closely related (Naether, 1939). Despite this branching structure seems to be reasonable in the view of such information, other individuals of these breeds are carrying different haplotypes. Also, other individuals coming from the Muzaffer Göl’s poultry are carry different haplotypes, Haplotype 1 and 2 meaning that the poultry is not homogenous in the means of haplotypes. So, both of these explanations need further support.

Haplotypes 22, 23 and 24 formed a clade with the posterior equals to 0.94; Haplotypes 26 and 7 formed a clade with the posterior probability equals to 0.91.

Haplotype 22, 23 and 24, all of these haplotypes include sequences obtained from GenBank. Haplotypes 22 includes one individual specified as the Fancy Pigeon (no specific breed information supplied) with the GenBank accession number

KP168712.1. For this individual, no information has been given about the sampling region but based on the location of the institute that researchers who wrote the paper are working, it can be deduced that sampling may be done in the Zhangjiakou city, Hebei region, China. Haplotype 23 includes a sequence having no breed information. GenBank accession number of the sequence is NC_013978.1. As per above, sampling may be done in Wuhu, China. Haplotype 24 also includes only one sequence belonging to the King Breed. GenBank accession number of this sequence is KP258178.1. As associated data was produced in Xuanhua, China, the sampling may have been done in the same region, too. If the reasoning above is valid, the reason of those samples is grouped together may be the geographical proximity of the sampled individuals.

Haplotype 26 includes two samples, having no breed information, with GenBank accession numbers JF498842.1 and GQ481605.1. The sample coded as JF498842.1 released under the headline “Public data release of DNA barcodes for birds of Hawaii” on GenBank. Therefore, the sample was probably taken from Hawaii. For the sample coded GQ481605.1, no sampling region information could have been found but, from the headline of the article, it can be understood that the sample was taken from somewhere in the Palearctic region (Kerr, Birks, et al., 2009). Since those two regions are unrelated and no breed information was given, no inference can be done on the coexistence of those individuals in the same clade.

Haplotype 7 includes both of the two Modern Oriental breed samples. It was mentioned that the Modern Oriental Breeds has thought to be developed by using the Classical Oriental Frill in several historical sources (Naether, 1939) (Wright, 1880) (MacLeod, 1913), which also was verified by most of the Turkish breeders. In the

Bayesian tree of *COI* haplotypes of Turkey, Modern Oriental Frill samples carry the same haplotype and this haplotype is separated from all of the Classical Oriental Frill samples.

Haplotypes 18, 19, 17 and 5 formed a clade having the posterior probability equal to 0.80. Haplotype 18 includes a highflier breed, Usak Highflier. Haplotype 19 includes Demirgech breed which is known with its significant singing. Haplotype 17 includes Enice breed which is a short-beaked owl breed. Haplotype 5 includes Macedonian Donek and Aydin breeds which are belonging to the spinner/roller group, Classical Oriental Frill which is an owl and Usak Highflier. None of these haplotypes are breed specific and this clade looks like a mixture clade of different types of breeds without any relation about breed features, maternal origins and poultry they were sampled from.

Although it was shown in many studies that *COI* is a successful marker in identifying bird species (Hebert, Stoeckle, Zemlak, & Francis, 2004) (Kerr et al., 2007) (Johnsen et al., 2010) and was also used for distinguishing different populations for some invertebrates, it is too conserved to be used as a marker for intraspecific studies of pigeons (Sonoda et al., 2011) (Biała, Dybus, Pawlina, & Proskura, 2015). Ramadan et al. also used *COI* as a marker for analysis of 11 pigeon breeds. Their Neighbor Joining tree also showed that all of the breeds are clustered together with *Columba livia* and formed just one clade. They concluded that *COI* is more suitable marker for interspecific analysis rather than intraspecific ones (Ramadan et al., 2018).

In conclusion, *COI* could not provide enough data to infer trustable conclusions and remained insufficient for construction of pigeon breed phylogeny.

3.4.2 Median Joining Network of *COI* Haplotypes

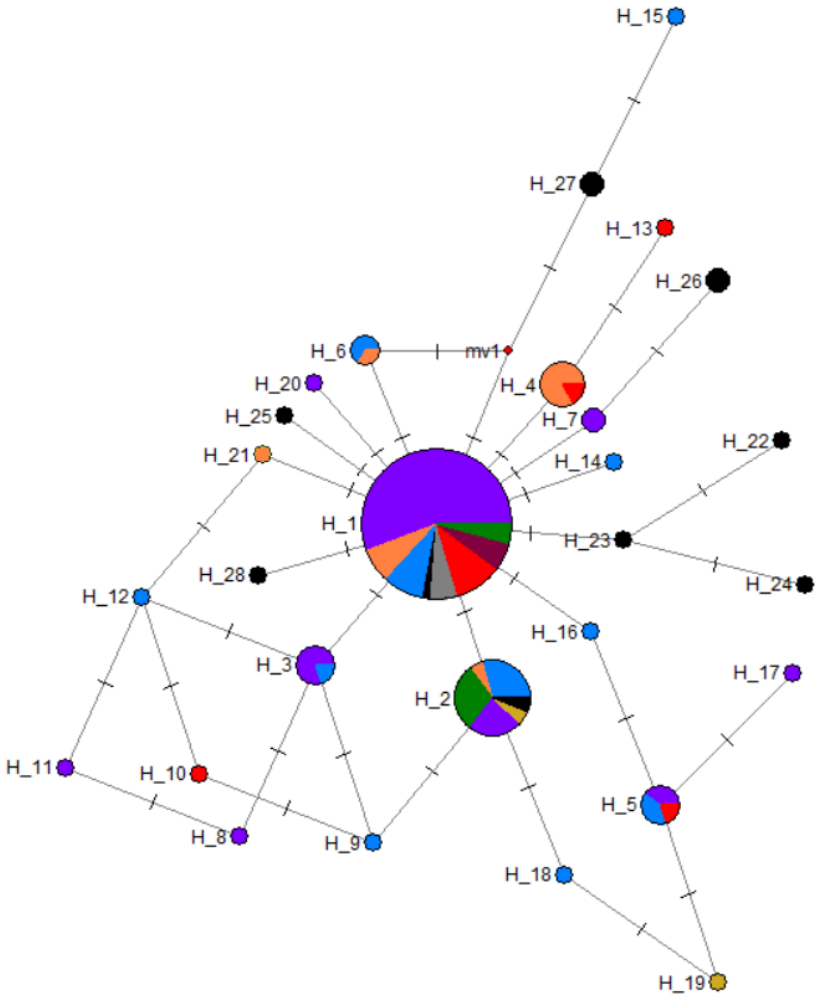

Figure 3-7 Median Joining Network (Bandelt, Forster, & Röhl, 1999) of unique haplotypes of naïve pigeon breeds in Turkey. Every line on the branches demonstrates the number of mutations between haplotypes that branch is connecting. Every breed group is shown with different color, according to the classification constructed in this study based on EFLP classification of Fancy Pigeons (European Association of Poultry Pigeon and Rabbit breeders, 2018) with the contribution of the breeder Murat Türkeş.

Table 3-5 Grouping of the pigeon breeds based on the EFLP grouping with the contribution of the breeder Murat Türkeş. Every breed group colored differently. These colors are used in the NETWORK of *COI* haplotypes.

Group Name	Breeds
Form Pigeons (1)	Romer (Runt) Adana Dewlap Damascene Egyptian Swift Syrian Swift
Color Pigeons (2)	Gimpel (Archangel) Nuremberg Lark
Trumpeter Pigeons (3)	Demirgech Pigeon
Structure Pigeons (4)	Chinese Owl Seldschuk (Selçuklu) Tavuskuyruk (Fantail)
Owl Pigeons (5)	African Owl Modern Oriental Frill (Modern Hünkari) Old/Classical Oriental Frill - Classical Oriental Frill

Table 3-5 (continued)

	- Manisa Azman - Tunisian Owl - Polish Owl
	Mısıri Pigeons
	- Misiri İzmir - Misiri Manisa - Enice - Misiri İstanbul - Bulgarian Bango
	Baska Pigeons
	- Classical Akbash Bango (Baska) - Akbaşh Bango (Akbash Baska)
	Denizli Azman
	Macar
	Tokur
Tumblers (6)	Szegedin Tumbler (or Highflier)
	Griwuni Tumbler
	Rostov Tumbler/Volski
	Uzbek Tumbler
	Botaschan Tumbler

Table 3-5 (continued)

	Malatya Tumbler
	Mosul Tumbler
	Shebap
Highfliers (7)	Usak Highflier
	Iran Highflier
	Budapest Highflier
	Pazarcik Highflier
	Denizli Katal
	Posta
Spinners and Rollers (8)	Thrace Roller
	Bursa Roller
	Saya
	Odemish
	Aydin
	Donek
	Dolapci
	Macedonian Donek
Feral / No breed information (10)	Rock Pigeon/Feral Pigeon

Most of the breeds belong the same, biggest and probably the oldest haplotype (Donelly & Tavare, 1986) (Watterson & Guess, 1977) (L. Excoffier & Smouse, 1994), Haplotype 1. This indicates that the domestication of pigeons probably occurred just once and other breeds are produced after that domestication event. Other haplotypes attached with the singletons to the bigger haplotypes tend to be the consequences of more recent mutational events. This star shaped network means that there is no or little geographical structure. Also the low level of sequence divergence and the high frequency of unique mutations can be the signatures of rapid population expansion (Slatkin & Hudson, 1991) (de Jong, Wahlberg, van Eijk, Brakefield, & Zwaan, 2011). Additional connections among the haplotypes can be the result of the animal transfer that breeders do to add new characteristics to their own breeds such as a new color, pattern or structures like crest or wattle. This result is also consistent with the phylogenetic tree above.

3.4.3 Bayesian Phylogenetic Tree of *Columba livia* D Loop Haplotypes

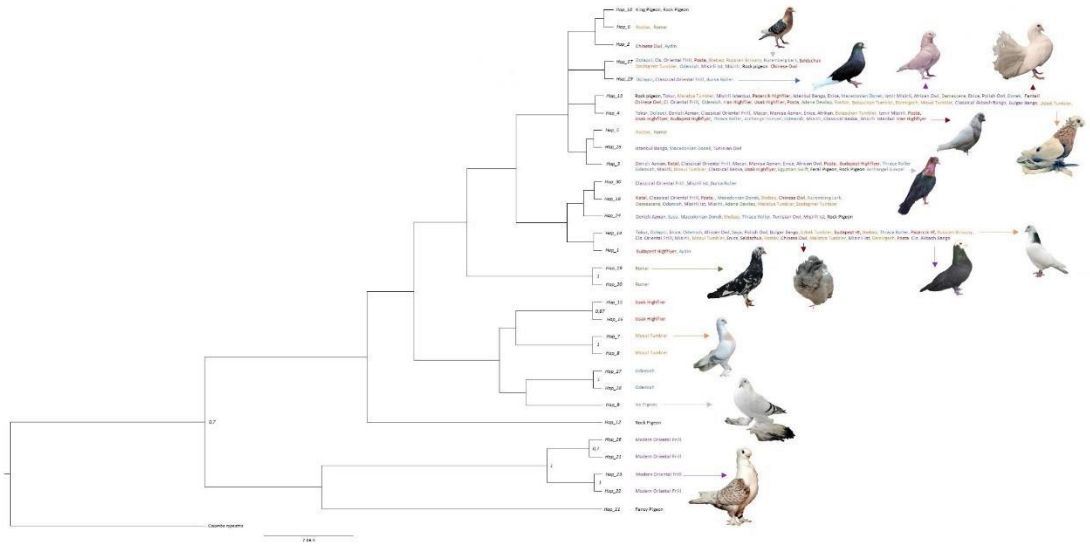

Figure 3-8 Bayesian Phylogenetic Tree of D loop Haplotypes. Numbers on nodes indicate the posterior probabilities of the branching structures. Colors indicate different pigeon groups based on the classification in Table 3-5.

Branch including haplotypes 26, 23, 21 and 22 has posterior probability equal 1. These haplotypes are carried by two individuals belonging to the breed Modern Oriental Frill and there is no other sample belonging to this breed among our samples. Historical records state that this breed was developed by using the Classical Oriental Frill breed taken from Smyrna to Europe in 1880s (Naether, 1939). Our finding has potential to support this historical information. This finding is also consistent with the finding of Bayesian analysis of *COI* region.

Haplotype 27 and 28, Haplotypes 7 and 8 and Haplotypes 19 and 20 formed branches that have the posterior probabilities equal to 1. Haplotypes 15 and 16 have the posterior probability equal to 0,87. All of these successive haplotypes are carried by same individuals, differing only in the number of thymines in the T stretch.

According to the results of this Bayesian phylogenetic tree, the D loop marker was not successful in differentiating distinct pigeon breeds in Turkey, except Modern Oriental Frill breed. Our results showed that D loop is a variable marker especially in terms of length polymorphism. However, it did not produce a good phylogenetic signal due to both high levels of heteroplasmy and also introgression among distinct breeds. In the Bayesian phylogenetic tree, haplotypes did not form a distinct clade to represent different breeds or breed groups, instead, they distributed evenly throughout the tree.

D loop have been used in different studies in *Columba* genus. In one of these studies, the genetic variations of the populations of the *Columba inornata* species living in two different regions were compared. It has been found that these are subspecies, genetically significantly different from each other and conservation strategies should be shaped accordingly. The haplotype variations of the subspecies taken from these two different geographical regions were 0.4791 and 0.5908, and the nucleotide variations were 0.2% and 0.4%. The haplotype diversity of our study was higher than this study due to high level of heteroplasmy seen in samples as described above. This study was a study showing that D loop is promising as a genetic marker (Young & Allard, 1997).

In another study, the genetic differences of the endangered *Columba bollii* and *Columba junoniae* species were investigated. DNA obtained from muscle, hair and stool samples were analyzed. Sequencing and RFLP techniques were applied to D loop PCR products. This study was the first study in which congeneric and sympatric bird species are molecularly differentiated. However, the results show that their D loop region contain only a few variable regions (Marrero, Cabrera, Padilla, & Nogales, 2008).

Seki (2006) studied the genetic variations of different pigeon species using the D loop. In this study, similar to ours, feathers were used as a source of DNA, but heteroplasmy was not reported. It has been indicated that the D loop marker was variable and successful for constructing informative phylogenetic trees.

368 bp long D loop region of different *Columba palumbus* populations were analyzed. Specific haplotype structures were defined for couple of different populations. But, in the phylogenetic tree drawn, bootstrap results were not high enough to make definite inferences. Similar to our case, one most common haplotype defined, and other haplotypes derived from the main haplotype by several mutations (Butkauskas, Švažas, Sruoga, Bea, & Grishanov, 2008). Unlike our study, natural living *Columba palumbus* populations were used in this study. These populations naturally live separately from each other. The individuals in our study are domestic and are likely to be traded continuously among breeders. This causes an increase in gene flow between breeds and results in a phylogeny which is hard to resolve at the breed-scale. In spite of being a promising marker for successfully detecting intraspecific variation and characterizing the Turkish pigeon breed populations, our data obtained from D loop was not sufficient to reveal the population structure of Turkish pigeon breeds.

There are only two studies in *Columba livia* species where D loop analyses have performed. The first study does not include a phylogenetic breed analysis (Tsai et al., 2009) (Lee, Tsai, Liao, Linacre, & Hsieh, 2010). In this study, a 61 bp VNTR region and a STR region (5'-(CAAA)n-3') in the D loop region were identified. It

was also observed that birds are heteroplasmic in terms of repeat numbers of STR. The purpose of the study is to measure whether the D loop can separate individual birds from different maternal backgrounds. This study was carried out in Taiwan in which gambling on pigeon races is common. In Taiwan, sometimes judicial problems occur in the races and it is needed to be confirmed the maternal origins of the pigeons raced. In other words, the purpose of grouping pigeon breeds is not intended. Rather, it is intended to verify, for example, poultries of raced pigeons. In this sense, it has been investigated whether D loop will be useful or not (Tsai et al., 2009).

In the second study, the VNTR and STR regions at the end of the *Columba livia* D loop were analyzed together with capillary electrophoresis and were found to have a high discrimination power (Lee et al., 2010). Haplotypes were created according to both SNPs, VNTR repeats and STR repeats. Similar to our results, the number of SNPs in D loop is very low here. Only 3 haplotypes could be detected. The number of VNTR repeats varies between 2 and 8. The most common haplotype includes 4 VNTR repeats. In our study, the most common haplotypes (Hap_13 and Hap_14) include 4 VNTR repeats, too. In this study, the VNTR region has also been shown to be heteroplasmic. Even it was detected that one individual carrying 4 different alleles at the same time. Unlike our study, combinations of different repeats in heteroplasmic individuals were also considered as separate haplotypes (Such as, carrying Haplotype A and Haplotype B together constructs another haplotype, Haplotype C). In this way, 21 different haplotypes were obtained. There is also an example in this study where heteroplasmy is transferred from mother to offspring (Lee et al., 2010). Likewise, the STR region is heteroplasmic as previously determined. Because of the heteroplasmy, the capillary electrophoresis peaks of the STR region cannot be separated (Lee et al., 2010). Finally, in this study haplotypes were created by combining VNTR repeats and SNPs and 38 haplotypes from 131 individuals were obtained in this way. However, different from our study, different combinations of heteroplasmic haplotypes with SNPs were evaluated as separate haplotypes (Such as, having haplotypes including 4 and 5 repeats together with a

particular SNP constructing another haplotype). As a result, it is concluded that the D loop can be used to separate maternal origins of pigeons (Lee et al., 2010).

When all these different studies considered together, the reasons for the failure of D loop in differentiating the pigeon breeds in Turkey can be listed as follow:

One of the possible reasons is the heteroplasmy found in pigeon breeds. A large number of pigeons in the study were carrying different mitochondrial molecules together. These mitochondria differed in the repeat number of 61 bp-length VNTR region and T number in the T stretch. This has prevented the construction of an accurate phylogenetic tree that could successfully separate pigeons in terms of their breeds, their geographical area, or their poultry. So, it can be deduced that the heteroplasmy present in the data set caused the suppression of phylogenetic signals.

Another reason may be the low number of samples per breed. This hypothesis seems to be compatible with the results of the haplotype accumulation curves. Another reason can be that the regions sampled are geographically close. The breeders living in these regions are also in close contact, which strengthens the chances of bird exchange among themselves. These may increase the gene flow among different breeds. As a result, if more samples are taken from each breed and from distant geographical regions, the results are likely to be more meaningful.

3.5 Historical Demography of *Columba livia* Population

3.5.1 Mismatch Distribution

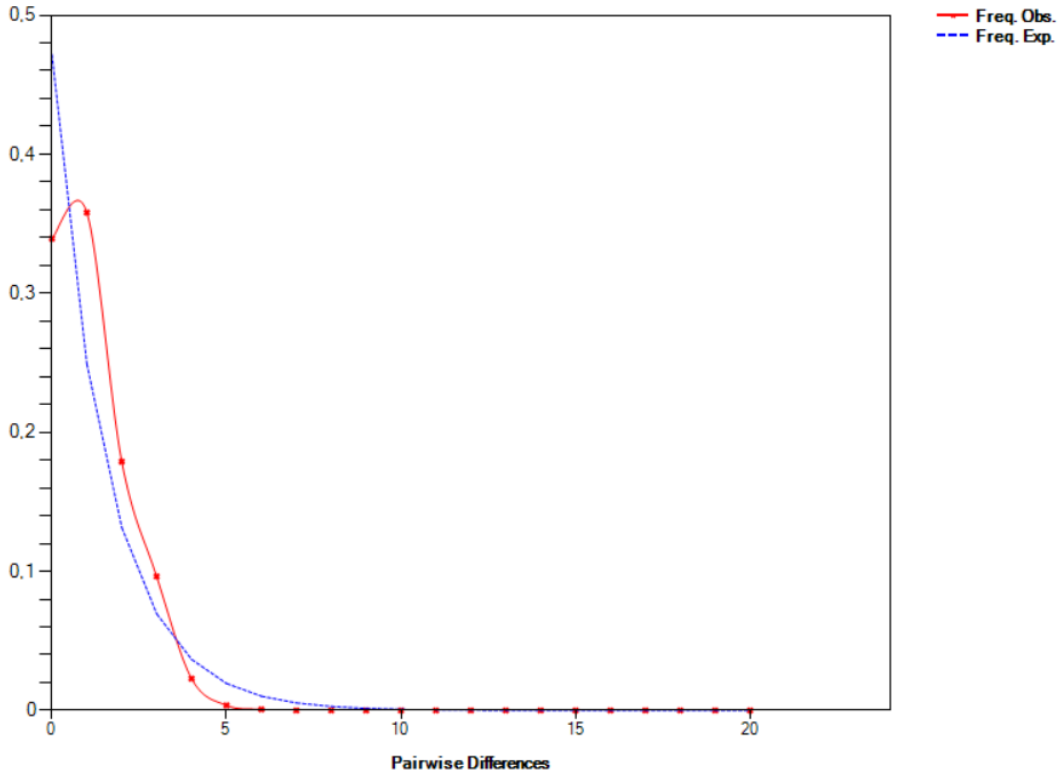

Figure 3-9 Mismatch distribution graph of *COI*. Model selected as constant population size. While the red line stands for the observed frequency of pairwise differences, the blue line stands for the expected frequency of pairwise differences.

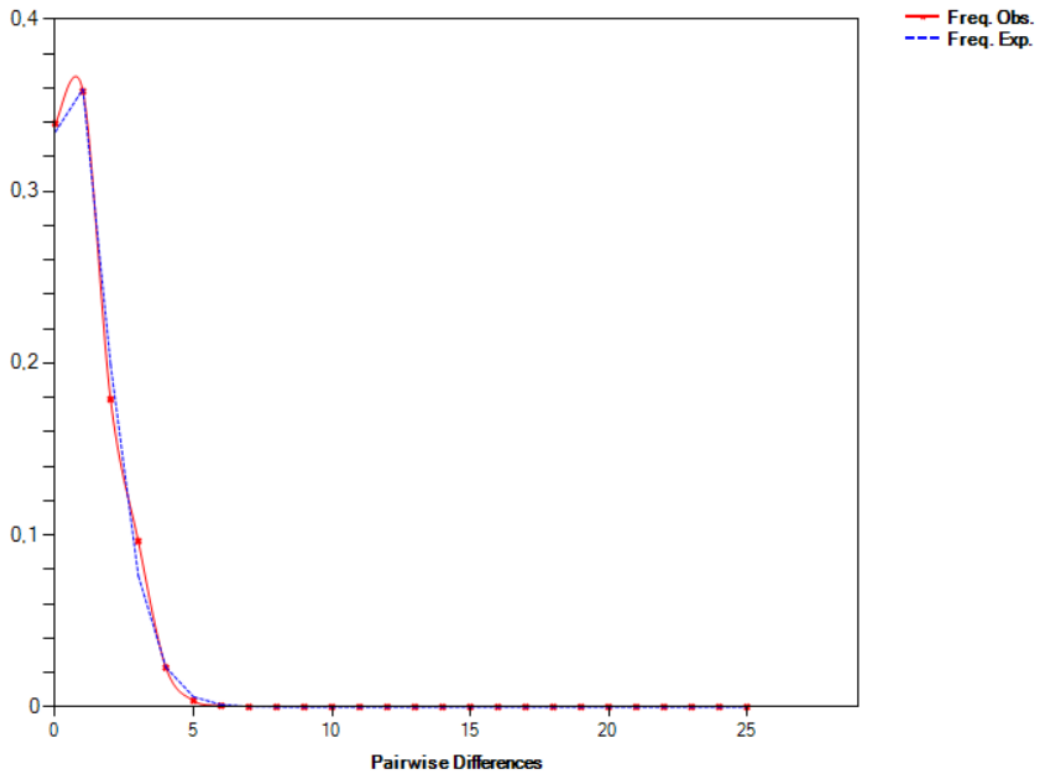

Figure 3-10 Mismatch distribution graph of *COI*. Model selected as population size growth/decline. While the red line stands for the observed frequency of pairwise differences, the blue line stands for the expected frequency of pairwise differences.

Mismatch distribution analysis (Rogers & Harpending, 1992) was done in DnaSP v.6 program by using constant population size model and changing population size model, respectively. Resulted graphs clearly showed that expected and observed mismatch distributions match each other when population growth model was chosen. Graphs resulted from the analysis by using two different models are given in the Figure 3-9 and Figure 3-10. Also, both of the figures demonstrate unimodal distribution indicating the population expansion (Rogers & Harpending, 1992) (Venkatesan, Westbrook, Hauer, & Rasgon, 2007). Raggedness statistics was 0.450 and not significant according to the result of coalescent simulation which was run 10.000 times in DnaSP v.6. Non-significant raggedness index and uncomplete unimodal curve of the mismatch distributions may be indicating that population growth occurred too recently and, therefore, couldn't produce a smooth mismatch

distribution (Harpending, 1994) (Bernard et al., 2014). Result of mismatch distribution is also consistent with the phylogeny of the *COI* with lots of external branches (Slatkin & Hudson, 1991).

3.5.2 Results of Fu and Li's Test, Tajima's D Test, Fu's Fs Test, Strobeck's Statistics

Tajima's D, Fu's Fs and Fu and Li's D and F are designed to observe the levels of deviations from neutrality which is the based on the constant population size at equilibrium. Deviations in these statistics state violations rather in constant population size or equilibrium, meaning that population size is changing or the region is under selection (Joshi et al., 2013) (Venkatesan et al., 2007). Results of these statistics are shown in Table 3-6.

Table 3-6 Summary Statistics of *COI* Gene Variation in *Columba livia* Breeds in Turkey

N	H	S	Hd	pi	K	D	Fs	S	D*	F*	r
115	21	13	0,661	0,0015	1,119	-1,543**	-19,131***	1***	-1,107	-1,391	0.045

N, number of sequences analyzed; H, number of haplotypes; S, number of segregating sites; Hd, haplotype diversity; p, nucleotide diversity; K, average number of nucleotide differences; D, Tajima's statistic (Tajima, 1989); FS, Fu's Fs test (Yun Xin Fu, 1997); S, Strobeck's statistic (Strobeck, 1987); D* and F*, Fu and Li's statistics (Y. X. Fu & Li, 1993), r, raggedness statistics (Harpending, 1994) , * P < 0.05; ** P < 0.01.; *** P < 0.001

Fu's Fs is resulted negative (-19,131, P < 0.001) when an excess of rare haplotypes is present. Negative Fu's Fs indicates the occurrence of population expansion or genetic hitchhiking (Yun Xin Fu, 1997) (Venkatesan et al., 2007). Negative values of Fu and Li's D* and F* (D* = -1,707, F* = -1,391) indicate the abundance of newly arisen haplotypes. It can be deduced from here that population expansion, genetic hitchhiking or background selection has taken place (Y. X. Fu & Li, 1993). The significant value of Fu's Fs together with non-significant Fu and Li's D* and F* are

evaluated as the absence of background selection by Fu (Yun Xin Fu, 1997). This finding supports the signals of demographic expansion or genetic hitchhiking (Yun Xin Fu, 1997) (Lopes, Miño, & Del Lama, 2007). Strobeck's S is the probability of obtaining equal or fewer haplotypes based on gene frequency and mutation rate (Strobeck, 1987). It is found to be equal to 1.000. Tajima's D is based on the average number of pairwise differences and the number of segregating sites (Slatkin & Hudson, 1991). The negative Tajima's D (-1,543, $P < 0.01$), reveals the abundance of low-frequency polymorphisms than expected and indicates either population expansion or positive selection (Tajima, 1989) (Joshi et al., 2013).

Overall, significant Fu's Fs together with non-significant Fu and Li's D and F and negative value of Tajima's D indicates that population of Turkish pigeon breeds has undergone a recent population expansion or genetic hitchhiking. Population expansion and genetic hitchhiking produces similar results and there is no efficient comparative test to separate them. (Ramos-Onsins & Rozas, 2002). So, both of these remain possible for Turkish pigeon breeds.

CHAPTER 4

CONCLUSIONS

DNA isolation from feathers and amplification were successful for both regions. 21 unique *COI* haplotypes and 26 unique D loop haplotypes were defined belonging to the *Columba livia* domestica breeds of Turkey. After adding GenBank sequences number of unique haplotypes increases to 28 for *COI* and 30 for D loop data sets. Haplotype diversity was h = 0.661 for *COI*, seems at moderate level. D loop haplotype diversity was h = 0.882 which is quite high even more than seen in *COI* gene due to heteroplasmy in the D loop region.

During amplification process of D loop, it was observed that D loop samples produce more than one band. These bands are close to each other, excluding the possibility of being non-specific amplification products. After sequencing those bands separately, it was revealed that D loop region is heteroplasmic in pigeons, including more than one mitochondrial variant. According to the results of Sanger sequencing, it was demonstrated that D loop is heteroplasmic for T stretches. Different haplotypes carrying 9 or 10 T repeats in the T stretch were observed. Also, it was shown that D loop has a 61 bp long VNTR region and different haplotypes carrying different number of repeats of this VNTR region were shown. Our findings are supported by two different studies. In these studies, heteroplasmy in domestic pigeons was demonstrated and 61 bp long VNTR region was characterized.

Haplotype Accumulation Curves were drawn to detect whether sampling is enough or not. The general acceptance is that a slope of 0.01 and below strongly proves that sampling is sufficient, and that a slope of 0.1 and above strongly proves that sampling is inadequate according to Phillips et al (Phillips et al., 2019). Estimated slopes of the curves were 0.12 and 0.065 for *COI* and D loop, respectively, meaning that in every

100 new samples approximately 12 new haplotypes can be discovered for *COI* and approximately 6.5 new haplotypes can be discovered for D loop. The *COI* curve does not tend to reach an asymptote. In the D loop, on the other hand, the number of haplotypes is high due to heteroplasmy. This much of sampling may not be sufficient for samples without heteroplasmy. While a clear threshold value was not accepted in this study to determine the satisfaction of the curve, the threshold values determined in other studies are generally arbitrary (Phillips et al., 2019). Besides, the functional forms of such curves are not fully known and these forms may differ considerably between taxonomic units (Phillips et al., 2019). In conclusion, genetic diversity studies based on these mitochondrial markers should include as many samples as possible to eliminate biased estimate of genetic diversity and other types of analyses.

Bayesian phylogenetic analyses have been done with both D loop and *COI* haplotypes. While outgroup sequences for *COI* gene were *Columba rupestris*, *Columba oenas* and *Columba palumbus palumbus*, for D loop only *Columba rupestris* was used as outgroup due to the absence of D loop sequences of other proper outgroup species. Both of the markers used could not provide enough data to infer trustable conclusions and remained insufficient for construction of pigeon breed phylogeny.

Median Joining Network was constructed with only *COI* sequences. Most of the breeds belong the same, biggest and probably the oldest haplotype (Donelly & Tavare, 1986) (Watterson & Guess, 1977) (L. Excoffier & Smouse, 1994), Haplotype 1. This indicates that the domestication of pigeons probably occurred just once and other breeds are produced after that domestication event. Other haplotypes attached with the singletons to the bigger haplotypes tend to be the consequences of more recent mutational events. This star shaped network means that there is no or little geographical structure. Also, the low level of sequence divergence and the high frequency of unique mutations can be the signatures of rapid population expansion (Slatkin & Hudson, 1991) (de Jong, Wahlberg, van Eijk, Brakefield, & Zwaan, 2011). Additional connections among the haplotypes can be the result of the animal

transfer that breeders do to add new characteristics to their own breeds such as a new color, pattern or structures like crest or wattle. This result is also consistent with the Bayesian phylogenetic trees.

Population consisting of pigeon breeds of Turkey subjected to different demographic analysis; mismatch distribution, Fu's and Li's Test, Tajima's D, Fu's Fs Test, Strobeck's Statistics. Resulted mismatch distribution graphs clearly showed that expected and observed mismatch distributions match each other when population growth model was chosen. Also, both of the graphs demonstrate unimodal distribution indicating the population expansion (Rogers & Harpending, 1992) (Venkatesan, Westbrook, Hauer, & Rasgon, 2007). Raggedness statistics was 0.450 and not significant. Non-significant raggedness index and uncomplete unimodal curve of the mismatch distributions may be indicating that population growth occurred too recently and, therefore, couldn't produce a smooth mismatch distribution (Harpending, 1994) (Bernard et al., 2014).

Fu's Fs and Fu and Li's D* and F* were negative demonstrating an excess of rare haplotypes and the occurrence of population expansion or genetic hitchhiking (Yun Xin Fu, 1997) (Venkatesan et al., 2007) (Y. X. Fu & Li, 1993). The significant value of Fu's Fs together with non-significant Fu and Li's D* and F* are evaluated as the absence of background selection by Fu (Yun Xin Fu, 1997). This finding supports the signals of demographic expansion or genetic hitchhiking (Yun Xin Fu, 1997) (Lopes, Miño, & Del Lama, 2007). Strobeck's S was found to be equal to 1.000. Tajima's D was negative revealing the abundance of low-frequency polymorphisms than expected and indicates population expansion, too (Tajima, 1989) (Joshi et al., 2013). Overall, significant Fu's Fs together with non-significant Fu and Li's D* and F* and negative value of Tajima's D indicates that population of Turkish pigeon breeds has undergone a recent population expansion or genetic hitchhiking. This finding is also consistent with the results of mismatch distribution analysis, Bayesian phylogenies and Median Joining Network.

By demonstrating the presence of high levels of haphazard gene flow among breeds, this study highlights the problems of pigeon breeding in Turkey. Indiscriminate crosses between different breeds and breed groups are apparently common place, resulting often in mitohaplotype transfers from an unrelated breed group to another, thus erasing the discriminatory power of genetic assignments. Also, the pigeon breeders in Turkey usually sell or give away the pigeons that they do not see valuable enough to cross with their "purebred" pigeons, leading to maintenance of birds with undesirable features in the greater gene pool. These practices of the Turkish breeder community might be one of the reasons for the disability of our approach to differentiate pigeon breeds and breed groups by phylogenetic analysis.

In conclusion, our results indicate that pigeon breeding in Turkey needs to be practiced in a more disciplined and deliberate manner if breeds such as Hünkari, surely a national heritage, can be kept for future generations. It would be useful that nationwide pigeon breeding associations and federations to actively cooperate and small associations to focus their activities on pigeon breeding instead of leisure activities.

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APPENDICES

A. Detailed list of all individuals in the study

Detailed list of all individuals in the study

Sequence Number	Breed Name	Origin	Location	Class	*COI* Haplotypes	D Loop Haplotypes
1	Tokur	Erkan Savran	Denizli - Pamukkale	Owl	Hap 1	Hap_4, Hap_13
2	Tokur	Erkan Savran	Denizli - Pamukkale	Owl	Hap 1	Hap_13, Hap_14
3	Tokur	Erkan Savran	Denizli - Pamukkale	Owl	Hap 1	Hap_13, Hap_14
4	Dolapci	Sırrı Yıldırım	Denizli - Merkezefendi	Spinner/Roller	Hap 1	Hap_4, Hap_17
5	Dolapci	Muhammet Çırak	Denizli - Pamukkale	Spinner/Roller	Hap 1	Hap_14
6	Dolapci	A. İhsan Otamış	Denizli - Pamukkale	Spinner/Roller	Hap 2	Hap_14, Hap_29
7	Dolapci	Dolapci	Denizli - Merkezefendi	Spinner/Roller	Hap 2	Hap_14, Hap_29
8	Denizli Azman	A. İhsan Otamış	Denizli - Pamukkale	Owl	Hap 1	Hap_4, Hap_24
9	Denizli Azman	A. İhsan Otamış	Denizli - Pamukkale	Owl	Hap 1	Hap_3, Hap_4
10	Katal	Sırrı Yıldırım	Denizli - Merkezefendi	Highflier	Hap 1	Hap_3, Hap_18
11	Classical Oriental Frill	İskender Damgacı	Denizli - Pamukkale	Owl	Hap 3	Hap_3, Hap_4
12	Donek	Sırrı Yıldırım	Denizli - Merkezefendi	Spinner/Roller	Hap 3	Hap_13

13	Macar	Hüseyin Eroğlu	Manisa - Salihli	Owl	Hap 3	Hap_3, Hap_4
14	İstanbul Bango	Hüseyin Eroğlu	Manisa - Salihli	Owl	Hap 1	Hap_13
15	Enice	Hüseyin Eroğlu	Manisa - Salihli	Owl	Hap 1	Hap_13, Hap_14, Hap_17
16	Classical Oriental Frill	Hüseyin Eroğlu	Manisa - Salihli	Owl	Hap 3	Hap_30
17	Manisa Azman	Hüseyin Eroğlu	Manisa - Salihli	Owl	Hap 3	Hap_3, Hap_4
18	Rostov	Serkan Gündüz	Manisa - Salihli	Tumbler	Hap 1	Hap_5, Hap_6
19	Classical Oriental Frill	Muzaffer Göl	Manisa - Salihli	Owl	Hap 11	Hap_18, Hap_29
20	Enice	Muzaffer Göl	Manisa - Salihli	Owl	Hap 8	Hap_3, Hap_4
21	Posta	Celal Erhan Barut	Manisa - Salihli	Highflier	Hap 10	Hap_17, Hap_18
22	African Owl	Ömer Bozkuş	İzmir	Owl	Hap 1	Hap_3, Hap_4
23	Botaschan Tumbler	Ömer Bozkuş	İzmir	Tumbler	Hap 21	Hap_4, Hap_13
24	Odemish	Mutlu Helvacı	İzmir - Kiraz	Spinner/Roller	Hap 12	Hap_13, Hap_14
25	Misirli İzmir	Ahmet Cila	Çanakkale - Umurbey Beldesi	Owl	Hap 20	Hap_4, Hap_13
26	Manisa Azman	Muzaffer Göl	Manisa - Salihli	Owl	Hap 1	Hap_3, Hap_4
27	Manisa Azman	Muzaffer Göl	Manisa - Salihli	Owl	Hap 1	
28	Classical Oriental Frill	Muzaffer Göl	Manisa - Salihli	Owl	Hap 1	

29	African Owl	Muzaffer Göl	Manisa - Salihli	Owl	Hap 1	Hap_13, Hap_14
30	Enice	Muzaffer Göl	Manisa - Salihli	Owl	Hap 1	Hap_4, Hap_13
31	Saya	Muzaffer Göl	Manisa - Salihli	Spinner/Roller	Hap 2	Hap_3, Hap_14, Hap_24
32	Posta	Celal Erhan Barut	Manisa - Salihli	Highflier	Hap 13	Hap_4
33	Polish Owl	Hüseyin Eroğlu	Manisa - Salihli	Owl	Hap 1	Hap_13, Hap_14
34	Bulgar Bango	Erol Osmanlı	Manisa - Salihli	Owl	Hap 1	Hap_13
35	Usak Highflier	Ali Arslan	Uşak - Merkez	Highflier	Hap 1	Hap_4, Hap_13
36	Uzbek Tumbler	Ömer Bozkuş	İzmir	Tumbler	Hap 2	Hap_13, Hap_14
37	Macedonian Donek	Ümit Ertürk	Uşak - Merkez	Spinner/Roller	Hap 1	Hap_13, Hap_18
38	Budapest Highflier	Ekrem Çavuş	Afyon - Sandıklı	Highflier	Hap 1	Hap_3, Hap_4
39	Budapest Highflier	Ekrem Çavuş	Afyon - Sandıklı	Highflier	Hap 1	Hap_1, Hap_14
40	Romer	Mehmet Demir	Manisa - Kula	Form	Hap 1	Hap_19, Hap_20
41	Romer	Mehmet Demir	Manisa - Kula	Form	Hap 1	Hap_5, Hap_6
42	Macedonian Donek	Ümit Ertürk	Uşak - Merkez	Spinner/Roller	Hap 1	Hap_24
43	Shebap	Ümit Ertürk	Uşak - Merkez	Tumbler	Hap 4	Hap_14, Hap_24
44	Shebap	Ümit Ertürk	Uşak - Merkez	Tumbler	Hap 6	Hap_17, Hap_18
45	Thrace Roller	Hüseyin Zabun	Manisa - Kula	Spinner/Roller	Hap 6	Hap_3, Hap_4

46	Thrace Roller	Hüseyin Zabun	Manisa - Kula	Spinner/Roller	Hap 14	Hap_14, Hap_24
47	Iran Highflier	Ali Arslan	Uşak - Merkez	Highflier	Hap 1	Hap_4, Hap_13
48	Bulgaria Pazarcik Highflier	Ali Arslan	Uşak - Merkez	Highflier	Hap 4	Hap_13
49	Grivuny Tumbler	Ersoy Koldaş	Avusturalya	Tumbler	Hap 1	Hap_14, Hap_17
50	Syrian Swift	Ersoy Koldaş	Avusturalya	Form	Hap 2	
51	Egyptian Swift	Ersoy Koldai	Avusturalya	Form	Hap 2	
52	Nuremberg Lark	Ersoy Koldaş	Avusturalya	Color	Hap 1	Hap_17, Hap_18
53	Gimpel Archangel	Ersoy Koldaş	Avusturalya	Color	Hap 1	Hap_3, Hap_4
54	Damascene	Ersoy Koldaş	Avusturalya	Form	Hap 2	Hap_13, Hap_18
55	Szedeginer Tumbler	Ersoy Koldaş	Avusturalya	Tumbler	Hap 1	Hap_17, Hap_18
56	Polish Owl	Hüseyin Eroğlu	Manisa - Salihli	Owl	Hap 1	Hap_13, Hap_14
57	Classical Oriental Frill	Hüseyin Eroğlu	Manisa - Salihli	Owl	Hap 1	Hap_3, Hap_4
58	Demirgech	Sertaç Adabay	İzmir - Tire	Trumpeter	Hap 2	Hap_13, Hap_18
59	Odemish	Mutlu Helvacı	İzmir - Kiraz	Spinner/Roller	Hap 1	Hap_3, Hap_4, Hap_17
60	Manisa Azman	Muzaffer Göl	Manisa - Salihli	Owl	Hap 1	Hap_3, Hap_4
61	Tunisian Owl	İsa Savaşkan	Çanakkale - Umurbey Beldesi	Owl	Hap 2	Hap_24

62	Classical Oriental Frill	Murat Türkeş	Çanakkale - Yapıldak köyü	Owl	Hap 1	Hap_13, Hap_14
63	Mısıri İstanbul	Murat Türkeş	Çanakkale - Yapıldak köyü	Owl	Hap 1	Hap_4, Hap_24
64	Mısıri İstanbul	Murat Türkeş	Çanakkale - Yapıldak köyü	Owl	Hap 1	Hap_17, Hap_18, Hap_30
65	Mısıri	Murat Türkeş	Çanakkale - Yapıldak köyü	Owl	Hap 1	Hap_3, Hap_14
66	Mısıri Manisa	Murat Türkeş	Çanakkale - Yapıldak köyü	Owl	Hap 1	Hap_17, Hap_18
67	Classical Akbash Bango	Murat Türkeş	Çanakkale - Yapıldak köyü	Owl	Hap 2	Hap_14
68	Classical Baska Bango	Murat Türkeş	Çanakkale - Yapıldak köyü	Owl	Hap 2	
69	Mısıri	Hüseyin Eroğlu	Manisa - Salihli	Owl	Hap 1	Hap_4, Hap_17
70	Mosul Tumbler	Mustafa Mert Yeşilkaya	Mersin - Toroslar	Tumbler	Hap 4	Hap_3, Hap_4, Hap_14
71	Mosul Tumbler	Mustafa Mert Yeşilkaya	Mersin - Toroslar	Tumbler	Hap 1	Hap_7, Hap_8, Hap_14
72	Mosul Tumbler	Mustafa Mert Yeşilkaya	Mersin - Toroslar	Tumbler	Hap 4	Hap_13
73	Enice	Hüseyin Eroğlu	Manisa - Salihli	Owl	Hap 1	Hap_13, Hap_14
74	Classical Oriental Frill	Ramazan Özdemir	Manisa - Salihli	Owl	Hap 1	Hap_3, Hap_4
75	Bulgar Bango	Erol Osmanlı	Manisa - Salihli	Owl	Hap 1	Hap_13, Hap_14

76	Modern Oriental Frill	Erol Osmanlı	Manisa - Salihli	Owl	Hap 7	Hap_21, Hap_26
77	Modern Oriental Frill	Erol Osmanlı	Manisa - Salihli	Owl	Hap 7	Hap_22, Hap_23
78	African Owl	Ömer Bozkuş	İzmir	Owl	Hap 1	
79	Manisa Azman	İskender Damgacı	Denizli	Owl	Hap 1	Hap_3, Hap_4
80	Adana	Ahmet Oyar	Adana	Form	Hap 2	
81	Adana	Ahmet Oyar	Adana	Form	Hap 1	Hap_13, Hap_18
82	Seldschuk	Muzaffer Bey	Konya - Selçuklu	Structure	Hap 1	Hap_14, Hap_17
83	Classical Akbash Bango	Murat Türkeş	Çanakkale Yapıldak köyü	Owl	Hap 1	Hap_13, Hap_14
84	Saya	Mehmet Demir	Türkiye	Spinner/Roller	Hap 9	
85	Volski	Yasir Arabacı	Denizli - Pamukkale	Tumblers/Highfliers	Hap 1	Hap_13, Hap_14
86	Fantail	Yasir Arabacı	Denizli - Pamukkale	Structure	Hap 1	Hap_13
87	Chinese Owl	Yasir Arabacı	Denizli - Pamukkale	Structure	Hap 1	
88	Chinese Owl	Yasir Arabacı	Denizli - Pamukkale	Structure	Hap 1	Hap_2, Hap_17
89	Malatya Tumbler	Yasir Arabacı	Denizli - Pamukkale	Tumbler	Hap 4	Hap_13, Hap_18
90	Malatya Tumbler	Yasir Arabacı	Denizli - Pamukkale	Tumbler	Hap 4	Hap_13, Hap_14
93	Bursa Roller	Ahmet Yağız	Bursa	Spinner/Roller	Hap 2	
94	Bursa Roller	Ahmet Yağız	Bursa	Spinner/Roller	Hap 6	Hap_29, Hap_30

104	Classical Oriental Frill	Murat Türkeş	Çanakkale - Yapıldak köyü	Owl	Hap 1	Hap_3, Hap_4
106	Classical Oriental Frill	Murat Türkeş	Çanakkale - Yapıldak köyü	Owl	Hap 1	Hap_13, Hap_14
108	Classical Oriental Frill	İsa Savaşkan	Çanakkale - Umurbey Beldesi	Owl	Hap 1	Hap_3, Hap_4
109	Classical Oriental Frill	İsa Savaşkan	Çanakkale - Umurbey Beldesi	Owl	Hap 2	
113	Classical Oriental Frill	Murat Türkeş	Çanakkale - Yapıldak köyü	Owl	Hap 5	
115	Classical Oriental Frill	Murat Türkeş	Çanakkale - Yapıldak köyü	Owl	Hap 5	
116	Classical Oriental Frill	Murat Türkeş	Çanakkale - Yapıldak köyü	Owl	Hap 1	
117	Macedonian Donek	Ümit Ertürk	Uşak - Merkez	Spinner/Roller	Hap 5	
133	Misirli İstanbul	Murat Türkeş	Çanakkale - Yapıldak köyü	Owl	Hap 1	Hap_13, Hap_14
134	Classical Baska Bango	Murat Türkeş	Çanakkale - Yapıldak köyü	Owl	Hap 1	Hap_3, Hap_4
156	Aydin	Mutlu Helvacı	İzmir - Kiraz	Spinner/Roller	Hap 1	
157	Aydin	Mutlu Helvacı	İzmir - Kiraz	Spinner/Roller	Hap 1	Hap_1, Hap_2
159	Usak Highflier	Ali Arslan	Uşak - Merkez	Highflier	Hap 5	Hap_3, Hap_4
160	Usak Highflier	Ali Arslan	Uşak - Merkez	Highflier	Hap 16	

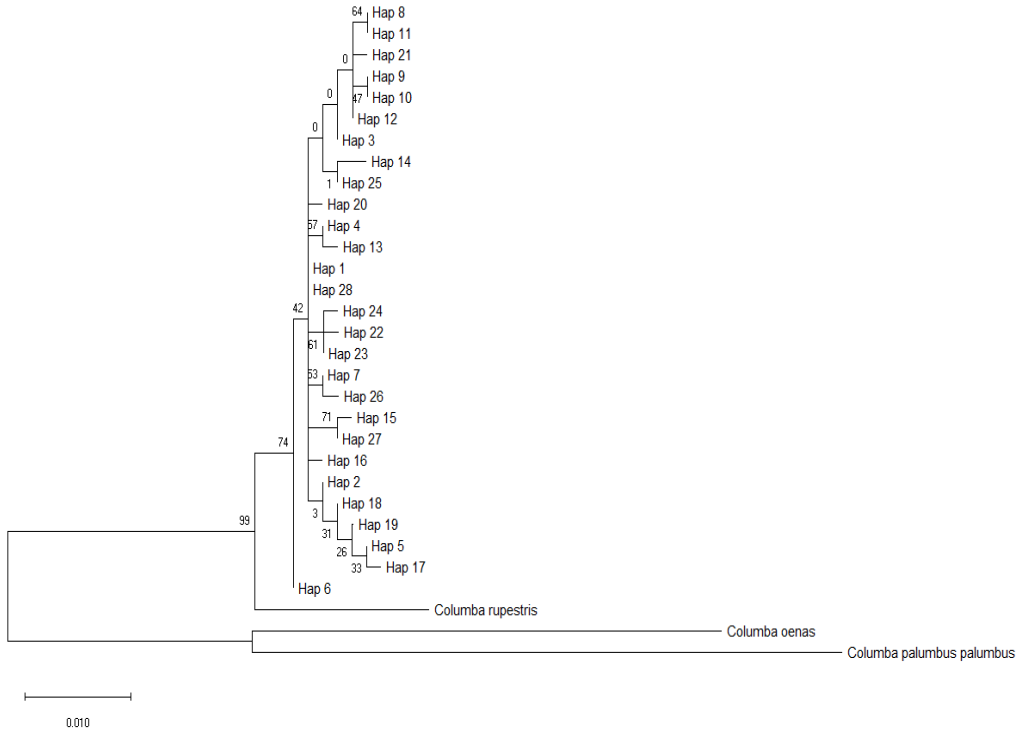
161	Usak Highflier	Ali Arslan	Uşak - Merkez	Highflier	Hap 18	Hap_15, Hap_16
162	Odemish	Mutlu Helvacı	İzmir - Kiraz	Spinner/Roller	Hap 2	Hap_27, Hap_28
163	Aydin	Mutlu Helvacı	İzmir - Kiraz	Spinner/Roller	Hap 5	Hap_1, Hap_2
164	Demirgech	Sertaç Adabay	İzmir - Tire	Trumpeter	Hap 19	Hap_13, Hap_14
167	Enice	Mustafa Kuzucuk	Manisa - Avşar	Owl	Hap 17	
168	Enice	Mustafa Kuzucuk	Manisa - Avşar	Owl	Hap 1	Hap_3, Hap_4
194	Saya	Mehmet Demir	Manisa - Kula	Spinner/Roller	Hap 15	
200	Posta	Ümit Ertürk	Uşak - Merkez	Highflier	Hap 1	
197	Posta	Ümit Ertürk	Uşak - Merkez	Highflier	Hap 1	Hap_13, Hap_14
	Archangel_Pigeon_KJ722068.1		Zhengzhou - China	Color		Hap_3
	Egyptian_Swift_KF926376.1		China	Form	Hap 2	Hap_3
	Feral_Pigeon_KF907308.1		China			Hap_3
	Rock_Pigeon_GQ240309.1		Taiwan			Hap_3
	Rock_pigeon_FJ792697.1_S5		Taiwan			Hap_3
	Rock_pigeon_FJ792695.1_S3		Taiwan			Hap_3

Rock_Pigeon_FJ792689.1_S1	Taiwan			Hap_3
Ice_Pigeon_KP306517.1	China	Color	Hap 1	Hap_9
King_Pigeon_KP258178.1	Xuanhua, China		Hap 24	Hap_10
Rock_Pigeon_NC_013978.1	Wuhu - China		Hap 23	Hap_10
Fancy_Pigeon_KP168712.1	Zhangjiakou city, Hebei region - China		Hap 22	Hap_11
Rock_Pigeon_KP319029.1	China			Hap_12
Rock_pigeon_FJ792690.1_S4	Taiwan			Hap_13
Rock_pigeon_J792696.1_S10	Taiwan			Hap_13
Rock_pigeon_FJ792693.1_S2	Taiwan			Hap_17
Rock_pigeon_FJ792692.1_S7	Taiwan			Hap_17
Rock Pigeon_FJ792691.1_S6	Taiwan			Hap_24
Rock_Pigeon_FJ792698.1_S8	Taiwan			Hap_24
Rock_Pigeon_FJ792694.1_S9	Taiwan			Hap_24

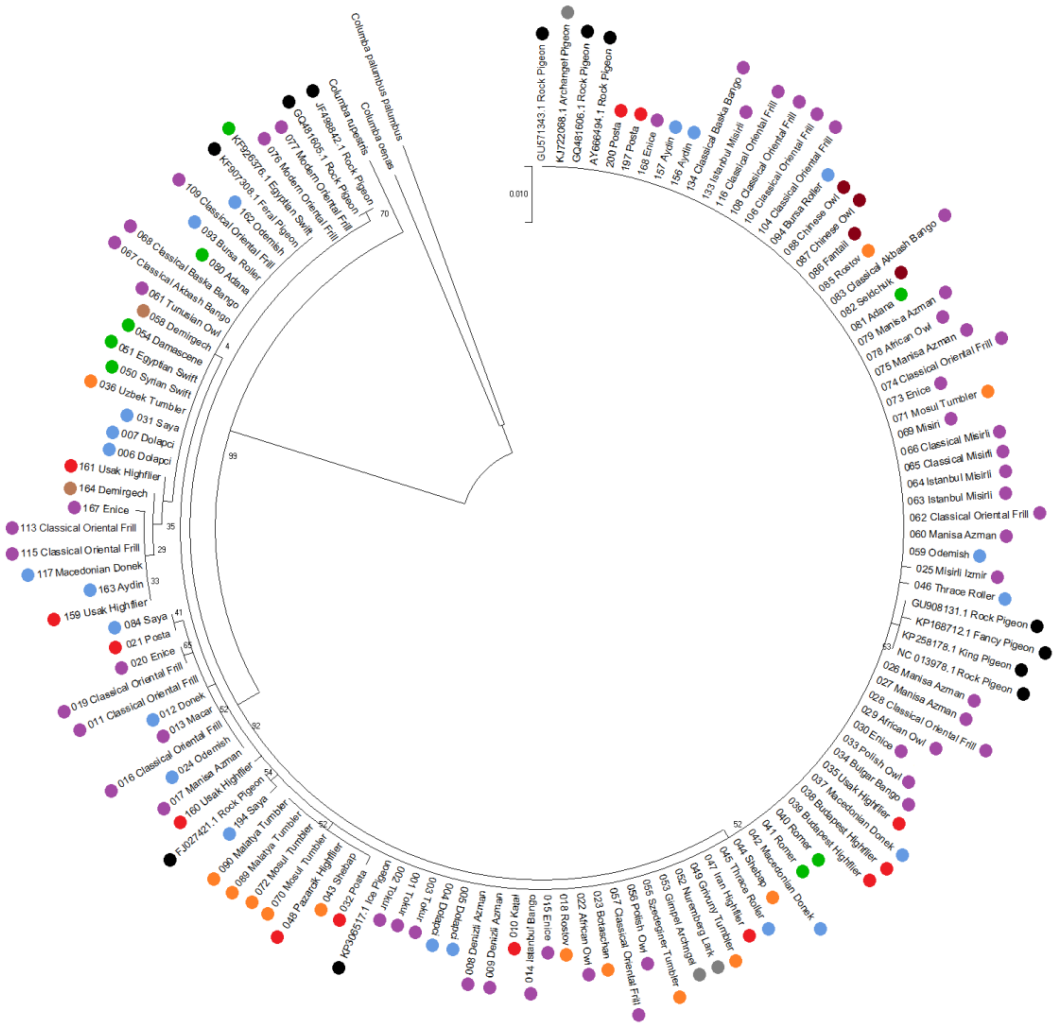
Rock_Pigeon_GQ481606.1	China	Hap 1
Rock Pigeon_GU571343.1	Scandinavia	Hap 25
Rock Pigeon_JF498842.1	Hawaii	Hap 26
Rock Pigeon_GQ481605.1	-	Hap 26
Rock Pigeon_FJ027421.1	Buenos Aires	Hap 27
Rock Pigeon_AY666494.1	North America	Hap 28

B. Maximum Likelihood Analysis of *COI* Haplotypes

Maximum Likelihood tree of the *COI* haplotypes was constructed in MEGA X (Kumar et al., 2018) software by using Hasegawa-Kishino-Yano (HKY) (Hasegawa, Kishino, & Yano, 1985) with gamma distributed site rate variation, using 4 discrete mutation classes (ln L= -1504.337, BIC= 3646.984).



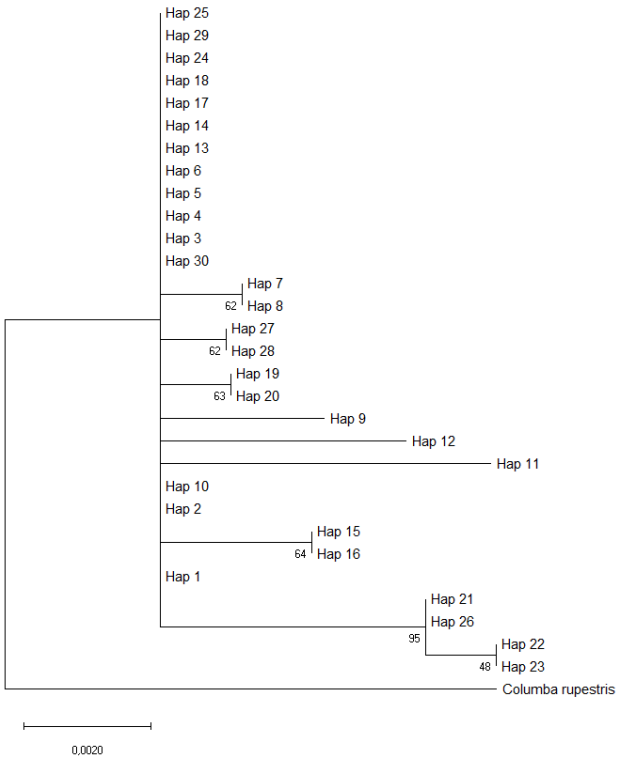
Maximum Likelihood tree of *COI* Haplotypes. Numbers on nodes indicate the bootstrap results of the branches. This tree was constructed in the MEGA X software.



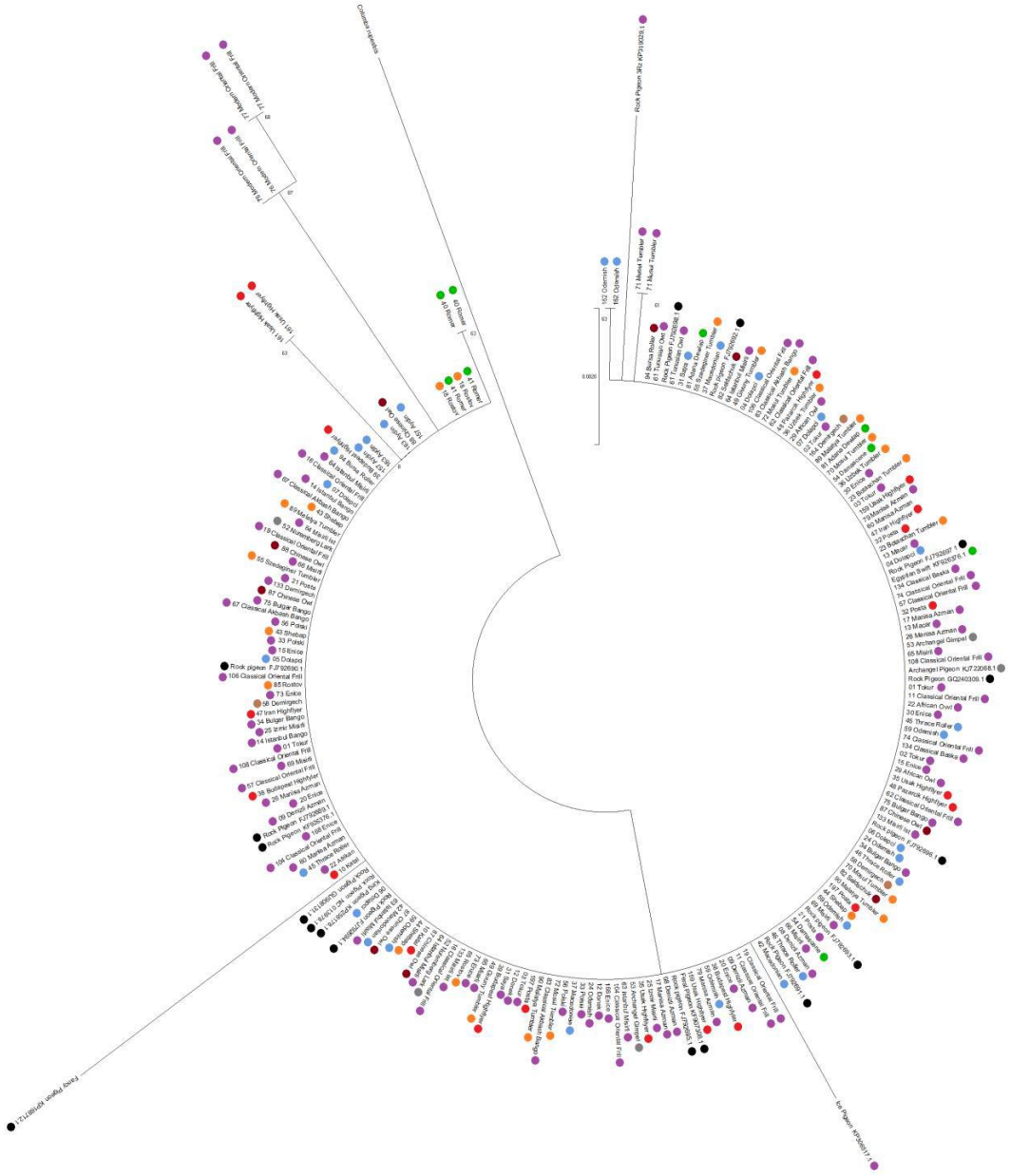
Maximum Likelihood tree of *COI* gene of all individuals. Numbers on nodes indicate the bootstrap results of the branches. This tree was constructed in the MEGA X software. Colored dots indicate different breed groups according to the grouping which is constructed based on ELFP grouping with contribution of the breeder Murat Türkeş (shown in Table 3-5).

C. Maximum Likelihood Analysis of D loop Haplotypes

Maximum Likelihood tree of the *COI* haplotypes was constructed in MEGA X (Kumar et al., 2018) software by using Hasegawa-Kishino-Yano (HKY) model (Hasegawa et al., 1985) (ln L=- -1679.61, BIC= 3718.92) with uniform site rate variation.



Maximum Likelihood tree of D loop region of all individuals. Numbers on nodes indicate the bootstrap results of the branches. This tree was constructed in the MEGA X software.



Maximum Likelihood tree of D loop region of all individuals. Numbers on nodes indicate the bootstrap results of the branches. This tree was constructed in the MEGA X software. Colored dots indicate different breed groups according to the grouping which is constructed based on ELFP grouping with contribution of the breeder Murat Türkeş (shown in Table 3-5).