CATALOGING THE GENETIC DIVERSITY OF BIRDS IN KARS-IGDIR REGION WITH GLOBAL COMPARISONS

by

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CATALOGING THE GENETIC DIVERSITY OF BIRDS IN KARS-IGDIR REGION WITH GLOBAL COMPARISONS

Loss of biodiversity, especially genetic diversity, is among the main problems of the modern times. Birds which are found nearly all habitat types are particularly sensitive to environmental changes. Caucasus endemic bird area that is partly located in Turkey is also threatened with habitat loss. More than 75% of all bird species recorded in Turkey are believed to be found in this region. In this study, DNA barcoding technique is used for the determination of genetic diversity of bird species in the Kars-Iğdır area. Seventy three COI sequences from 33 different species and 26 different genera are newly generated for this study. 301 sequences are added from the Barcoding of Life Database. The mean intraspecific divergence for Iğdır samples was 0.62%. From 12 species, 18 new haplotypes are recorded from Iğdır samples. 26 of 33 bird species analyzed in this study had unique barcode sequences that are distinct from those found in any other species in the BOLD database. Seven species have shared or overlapping barcode sequences. Using the sequences obtained in this study, global phylogeographic comparisons are made.

KARS IĞDIR BÖLGESİNDEKİ KUŞLARIN GENETİK KATALOGLANMASI

Biyolojik çeşitliliğin, özellikle de genetik çeşitliliğin kaybı son yüzyılın en önemi sorunlarından biridir. Neredeyse tüm habitat türlerinde bulunmakta olan kuşlar, çevresel değişiklilere karşı özellikle hassastırlar. Kafkas endemik kuş bölgesinin bir kısmı Türkiye'de bulunmakta ve habitat kaybından ciddi olarak zarar görmektedir. Türkiye'de bulunan kuş türlerinin %75'inden fazlasının bu bölgede bulunduğu düşünülmektedir. Bu çalışma Kars Iğdır bölgesindeki kuşların genetik çeşitliliğini DNA Barkodlama tekniği ile belirlemeyi hedeflemektedir. Otuz üç farklı tür ve 26 farklı cinsten 73 mitokondriyal COI bölgesi elde edilmiştir. BOLD veritabanından 301 farklı bireye ait COI bölgeleri incelenmiştir. Iğdır örneklerinin ortalama tür içi farklılığı 0.62% olarak tespit edilmiştir. On iki farklı türden 18 yeni haplotip kaydedilmiştir. İncelenen 33 türün 26'sının kendi türüne özgü BOLD veritabanındaki herhangi bir türden farklı bir barkoda sahip olduğu gözlemlenmiştir. Kalan yedi türün ise başka türlerle kesişen veya paylaşılan barkodları olduğu gözlemlenmiştir. Bu türlerin dizileri kullanılarak global filocoğrafi karşılaştırmalar da yapılmıştır.

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ABBI	All Birds Barcoding Initiative
BOLD	Barcode of Life Database
bp	Base pair
CBOL	Consortium for the Barcode of Life
Cytochrome c oxidase I	COI, CO1, cox1
DNA	Deoxyribonucleic Acid
DNTP	Deoxyribonucleotide triphosphate
EBA	Endemic bird area
EDTA	Ethylenediaminetetraacetic acid
Hap	Haplotype
IBA	Important bird area
IUCN	World Conservation Union
mtDNA	Mitochondrial deoxyribonucleic acid
PCR	Polymerase chain reaction
TBE	Tris base boric acid, EDTA

1. INTRODUCTION

1.1. Importance of Biodiversity, Biodiversity of Birds and General Threats

According to United Nations Convention on Biological Diversity, biological diversity or biodiversity is used to define the variety of life on Earth. The biodiversity is a consequence of billions of years of evolution, caused by natural processes. The convention aims to conserve biodiversity both for ethical and economic values. Biodiversity provides goods and services that help sustain our lives.

Biodiversity has components including ecological diversity, species diversity, and genetic diversity (Reaka-Kudla et al., 1997). There are genetic differences between and within species. Genetic diversity refers to the diversity within species. It is the main reason of the uniqueness of each individual and each species. Genetic diversity is important since it determines the ability of a population to tolerate different environmental conditions and stressors like droughts or parasites. So, high genetic diversity increases survival likelihood of species.

Birds are found in all habitat types and are very sensitive to environmental changes. Therefore, they are very good indicator species for monitoring the environment's healthiness (Gernant, 1997). According to the World Conservation Union (IUCN) Red Data List, there are 9990 bird species in the world. Of these, 134 of them are extinct and four of them are extinct in wild. 190 of birds are critically endangered, 361 are endangered and 671 are classified as vulnerable. 835 species are listed as near threatened. Main causes of declines in many bird species include the destruction and fragmentation of their habitats (Rubio et al., 2009) Decreases in wetland and grassland areas also threaten many bird species. Pesticides and other toxic chemicals are also causing declines in bird populations. Moreover water birds are threatened by oil spills, which are thought to be responsible for declines in seabird populations (IUCN, 2007).

Additionally, illegal bird trade has caused severe damage on many threatened species. Invasive species are also damaging to bird populations. Particularly island species are vulnerable to such introduced species, since many island birds have evolved in the absence of predators. For instance, flightless species, cannot cope with introduced species. As a result, nearly 75 percent of all bird extinctions occurred on islands. The IUCN reports that invasive species represent the single most frequent cause of bird extinctions since 1800.

Climate change is another threat for all avianfauna, and especially the species living in colder habitats with small range sizes have greater risk for extinction. Warming temperatures force many species to move to higher altitudes, reducing their ranges. Especially, when there is no habitat in higher elevations, extinction risk becomes even higher. Birds living in hotter habitats are more adapted to warmer temperatures, and can tolerate global warming better (Sekercioglu et al., 2009).

1.2. Importance of Turkey for Bird Life

Bird Life International has recognized more than 7500 important bird areas (IBAs) and 116 of them are in Turkey. IBAs programme applies a site based conservation approach to protect biodiversity and has two main selection criteria: vulnerability and irreplaceability. So, selected IBAs all have viable populations of birds that are either threatened or geographically concentrated.

Much of biodiversity has evolved in small areas of the world's surface known as “centers of endemism”. These unique places are particularly vulnerable to the destructive anthropogenic effects. The areas of avian centers of endemism are called “Endemic Bird Areas” (EBAs) by Birdlife International. There are 218 EBAs in the world and 26% of all bird species on earth are living in these areas, which cover only 5% of the world's land surface. Turkey has one EBA: Caucasus endemic bird area. Caucasus EBA is listed as one having high priority with major habitat loss. There are six countries in this region: Armenia, Azerbaijan, Georgia, Iran, Russia and Turkey.

One of these important spots, Aras-Iğdır Biodiversity region, covers Kars and neighboring cities (Ağrı, Ardahan, Artvin and Iğdır). Kars is at the north-eastern corner of Anatolia where the Iran-Anatolian and Caucasus biodiversity hotspots meet (Fig. 1.1). This area of Turkey contains 11 important plant areas, 13 important bird areas and 22 key biodiversity areas. More than 300 of the 465 bird species recorded in Turkey are believed to be found in this region (Birdlife International, 2009).

Figure 1.1. Map of Aras-Iğdır biodiversity region. Red triangle shows Aras ringing station.

1.3. Genetic Cataloging - DNA Barcoding

Mitochondrial DNA (mtDNA) has been widely used in phylogenetic studies since it evolves much rapidly than nuclear DNA. This results in the accumulation of differences between closely related species. Since, sequence divergences are larger among species than within species, by using mtDNA sequences, different species can be recognized. If a short region of mtDNA, that always differentiates species, could be found and accepted as a standard region, this region can be used as an identifier for species, shortly a ‘‘DNA barcode’’ (Herbert et al, 2004).

DNA sequences are major sources for improving our understanding of evolutionary and genetic relationships (Hajibabaei et al., 2007). The term DNA barcodes was first used in 1993, however it started to be more widely discussed in 2003 (Valentini et al., 2008). Genetic cataloging or DNA barcoding is based on the premise that a short standardized

sequence can differentiate individuals of a species from other species because genetic variation between species is more than that within species. The main aims of DNA barcoding are to allocate unknown specimens to species and improve the discovery of new species and help their identification. Studies showed the effectiveness of this method in several groups of animals, like birds, fish, and spiders. Furthermore, DNA barcoding systems are now being used for other groups of organisms, including plants, fungi and bacteria (Herbert et al., 2003). By DNA barcoding, a number of probably cryptic species, which were previously thought to be a single species, have also been discovered (Hajibabaei et al., 2005)

DNA barcoding starts with finding of the specimen to be investigated, using the proper preservation technique on the field to conserve its DNA until being transported to the laboratory. Laboratory protocols include isolation of DNA which is followed by PCR amplification, and sequencing (Herbert et al., 2003). Species identification through barcoding is usually done by using a short DNA sequence from a standard part of the genome. The barcode sequence is then compared with a library of reference barcode sequences. A specimen is identified, if its sequence closely matches one in the barcode library. Otherwise, the new record can lead to a new barcode sequence for a given species, or it can show the existence of a newly encountered species. For DNA barcoding in animals, mitochondrial gene cytochrome c oxidase I (COI, cox1) (648 base pair region 58–705 from the 50-end of the cytochrome c oxidase 1) is used (Hajibabaei et al., 2005). Studies show that more than 95% of species possess unique COI barcode sequences, so species-level identifications can be done successfully (Herbert et al, 2004).

The ideal DNA barcoding system should meet certain criteria (Frezal et al., 2008). These include:

1. Chosen gene region should be nearly identical among individuals of the same species, but different between species. Mitochondrial DNA has been used for DNA barcoding because it evolves more rapidly than nuclear DNA, so genetic differences between closely related species can be studied, even if they have been separated recently (Taberlet et al., 2006).

2. It should be standardized, with the same DNA region being used for different taxonomic groups.
3. Chosen DNA region should contain enough phylogenetic information to allocate unknown species to their taxonomic group.
4. It should have highly conserved priming sites and highly reliable DNA amplification and sequencing.

Main goal of DNA barcoding is to develop a standardized, rapid and inexpensive species identification method, which is also available to non-specialists (Hajibabaei et al., 2005). However, although DNA barcoding is a quick and relatively easy method for species identification, it has some disadvantages, which have to be taken into account. Firstly, sequencing is quite expensive. Secondly, some organisms, like certain plants do not have a COI gene, so it is not applicable to use COI gene for all taxa. Also hybrids can be difficult to categorize with this method (The New Zealand Biotechnology Learning Hub, 2009).

Considering birds, one of the important barcoding studies that included 260 bird species from North America indicated that all species had different barcodes and none of them shared between species. Additionally, COI differences even between closely related species were 18-fold higher than within species differences except a few species (Herbert et al, 2004).

A barcoding study by Yoo et al. (2006) revealed that 92 Korean bird species all have different COI sequences and barcodes from same species were either identical or very similar. According to the study the COI sequence differences between closely related species were 25-fold higher than the differences within species. In Scandinavian species, 94% of 296 species displayed unique barcodes (Johnsen et al., 2010). The remaining 6% had overlapping barcodes. Four species showed large intra-specific divergences within Scandinavia, although there is no morphological differentiation or reproductive isolation.

1.4. Species Accounts

Now a species-by-species overview of the subspecific designations, including details of genetic differentiation, if such data is available, will be given for the species investigated in this study. At the species level *Caprimulgus europaeus, Cuculus canorus, Emberiza schoeniclus, Erithacus rubecula, Ficedula parva, Luscinia svecica, Muscicapa striata, Parus major, Phoenicurus phoenicurus, Saxicola maurus* and *Sylvia curruca* exhibit high intraspecific divergence.

European nightjar *(Caprimulgus europaeus)* is distributed throughout northern and central Europe and Asia. It winters in Africa and has six recognized subspecies. These subspecies are classified according to morphology. As a result, *Caprimulgus europaeus europaeus* and *Caprimulgus europaeus plumipes* are as genetically divergent as different species from the same family (Larsen et al., 2006).

Common Cuckoo (*Cuculus canorus*) has four recognized subspecies. Three of the four subspecies winter in Africa. *Cuculus canorus* is a brood parasite so lays its eggs in the nests of other bird species. Since cuckoos use different species as hosts, they evolve different gentes to mimic eggs of hosts. As a result, the risk of the eggs being rejected by the hosts is reduced. Gentes are restricted to female lineages; since males are genetically identical, the common cuckoo remains genetically as one species. Studies show that there is differentiation between gentes in maternally inherited mitochondrial DNA, but not in microsatellite loci of nuclear DNA (Gibbs et al., 2000).

The reed bunting *(Emberiza schoeniclus)* is a passerine bird distributed in Europe and Asia. *Emberiza schoeniclus caspia* and *Emberiza schoeniclus intermedia* subspecies are observed in Turkey. Evolutionary mechanisms can be observed simply by using this species because it has a wide, but fragmented distribution and extreme levels of polymorphism (Matessi et al., 1999). For instance, this species shows large intraspecific polymorphism in bill size. Large billed birds have bills that are twice deeper than small billed ones. About 30 subspecies have been described, based on bill size and coloration. On the other hand, the variation of cytochrome-*b* genes in reed buntings has a mean divergence less than 0.5%, which is similar to what is observed within subspecies. So,

morphological variation is not reflected in the genetic structure of the populations (Grapputo et al., 1998).

The red-breasted flycatcher *(Ficedula parva)* and the spotted flycatcher, (*Muscicapa striata)* are passerine birds which breed in Europe and Asia. According to studies, the spotted flycatcher is the most divergent member of its family (Saetre et al., 2001). Based on mitochondrial DNA studies, *Ficedula parva albicilla,* which was previously accepted as a subspecies of *Ficedula parva,* is now named *Ficedula albicilla,* since it shows a high level of sequence divergence (Zink et al., 2008).

The great tit (*Parus major*) is a passerine bird. It is common throughout Europe, the Middle East, Asia, and North Africa. *Parus major* has four main subspecies (*Parus major major, Parus major minor, Parus major cinereus, Parus major bokharensis*). Totally there are 30 recognized subspecies. According to studies, during the coldest period of last glaciation, suitable habitats for great tits were present only in the southern parts of Europe. Six allospecies support the existence of several isolated refuges. According to mtDNA there is only one common pattern which can indicate ancestral *Parus major major* had one refuge in the last glacial period (Kvist et al., 1999).

The common redstart (*Phoenicurus phoenicurus*) and The European robin (*Erithacus rubecula*) are passerine birds. They were formerly classified in the Thrush family, but they are now classified in Muscicapidae. *Phoenicurus phoenicurus* shows relatively high (2%) intraspecific sequence variation (Johnsen et al., 2010), with no indication for cryptic species. There is no known morphological differentiation between these haplotype groups, and also there is direct evidence for interbreeding of the haplotypes (Kerr et al., 2009).

The Siberian stonechat (*Saxicola maurus*) and the bluethroat (*Luscinia svecica*) are now classified in the Muscicapidae family. They were formerly classified in Turdidae. They breed in temperate Asia and Europe. The common stonechat *(Saxicola torquata)* was previously considered as a single species with many European, Asian, and African subspecies. Currently, some authors recognize a single widespread species whereas others hypothesize the existence of at least six species. Recent studies on mtDNA sequences

support the recognition of the European stonechat (*Saxicola torquata rubicola*), the Siberian stonechat (*Saxicola maura maura*), the African stonechat (*Saxicola torquata axillaris*), the Reunion stonechat (*Saxicola (torquata) tectes*), the Madagascar stonechat (*Saxicola (torquata) sibilla*), and the Canary Islands stonechat (*Saxicola dacotiae*) as valid species based on differences in distribution, morphology and habitat preference (Zink et al., 2009). *Saxicola maurus armenicus* is a subspecies of Siberian stonechat which is found in the mountains of east Turkey. *Luscinia svecica* has 11 subspecies, one, *Luscinia svecica manga,* found in Caucasus area, eastern Turkey and Iran.

The lesser whitethroat (*Sylvia curruca*) is a widespread warbler that breeds in Europe and in western and central Asia. It is a strongly migratory passerine bird wintering in Africa. Two subspecies are recognized for the lesser whitethroat, and they meet at Central Europe. The western lesser whitethroat *(Sylvia curruca curruca*) is observed in western parts of range and northeastern lesser whitethroat (*Sylvia curruca blythi)* is observed in eastern parts of the species range.

As another group of species, *Acrocephalus palustris, Alcedo atthis, Cettia cetti, Coturnix coturnix, Coracias garrulus, Emberiza calandra, Emberiza citrinella, Emberiza hortulana, Galerida cristata, Lanius minor, Locustella luscinioides, Merops apiaster, Oenanthe hispanica, Oriolus oriolus, Passer domesticus, Passer montanus, Phylloscopus trochilus, Remiz pendulinus, Saxicola rubetra, Sylvia atricapilla, Sylvia nisoria* and *Turdus merula* show little intraspecific divergence.

The marsh warbler (*Acrocephalus palustris)* and the Savi's warbler (*Locustella luscinioides*) breed in temperate Europe and western Asia. The willow warbler (*Phylloscopus trochilus*) has a comparatively larger range, from Ireland to eastern Siberia. They all winter mainly in South Africa. The marsh warbler is monotypic, and no geographical variation is observed within species (Leisler et al., 2007).

The common kingfisher (*Alcedo atthis*) is a widely distributed bird, found across tropical Africa and Asia, Europe and temperate Asia. It is usually resident; it only migrates from places where rivers freeze in winter. Moyle et al. (2007) indicated that this species exhibits almost no sequence divergence, even between samples collected from France,

Mongolia, and Sulawesi. This fact is interesting since there are seven recognized subspecies of *Alcedo atthis*.

Cetti's warbler (*Cettia cetti*) and The European penduline tit (*Remiz pendulinus*) are passerine birds which breed in central Europe, northwest Africa and temperate Asia. Studies indicate that Cetti's warbler is expanding its range (Tasinazzo et al., 1993). This species spread from the Mediterranean coasts through western France over many countries of central and northern Europe. It recently started to breed in Britain. Similarly the European Penduline tit populations are also increasing.

The common quail (*Coturnix coturnix*) is a widespread bird and breeds throughout most of central and southern Europe, and in North Africa, including the Atlantic Islands close to Africa, and Europe. The evolutionary relationships and taxonomic status of European and East Asian populations of quail are still controversial. European and Far Eastern Japanese quails have been either considered as distinct (allo) species, or as two subspecies, namely the common quail *Coturnix coturnix coturnix* and the Japanese quail *Coturnix coturnix japonica.* Although the breeding distributions of these quails are mostly allopatric, according to Barilani et al. (2005) there are small areas of sympatry in the Baikal region in Russia and in the Kentei region in Mongolia. Experimental studies indicate that Japanese and Common quails do not have obvious pre-zygotic or post-zygotic reproductive isolating mechanisms, which support the notion that they should be accepted as subspecies (Barilani et al., 2005)

The European roller (*Coracias garrulus*) is found in Europe, the Middle East, Central Asia and Morocco. The lesser grey shrike (*Lanius minor*), breeds in southeastern Europe and Asia. They both winter in South Africa. The European roller populations are decreasing across its range in the Palearctic region. Since the second half of the eighteenth century, it has not been breeding in Denmark, Finland, Sweden, and eastern Germany (Avilles et al., 1999). Similarly abundance of the lesser grey shrike populations in Europe has declined in the recent years. Climate change and agriculture are considered to be the main reasons of this decline (Kristin et al., 2000).

The corn bunting (*Emberiza calandra* or *Miliaria calandra*) is a passerine bird in the bunting family Emberizidae. It breeds across Europe, North Africa and Asia all the way

to Kazakhstan. It is usually resident, but birds from colder regions of central Europe and Asia migrate southwards in winter. Some taxonomists recently placed it in a new genus *Miliaria*, whereas others believe that it belongs to the large genus *Emberiza*. The corn bunting, unlike *Emberiza* species, shows sexual dimorphism in size but not in plumage. Juveniles undergo a complete post-juvenile molt. For these reasons, some authorities put the corn bunting into a separate genus (*Miliaria*). On the other hand, the cytochrome-b data show that the corn bunting belongs to the *Emberiza* clade (Grapputo et al., 2001).

The yellowhammer (*Emberiza citrinella*) is a passerine bird. It breeds across Europe and Asia. It is mostly resident, but some birds from colder regions migrate south in winter. Studies show that although the yellowhammers and the pine buntings (*Emberiza leucocephalos)* differ obviously in appearance and song patterns, they have closely related mtDNA but not nuclear DNA. They hybridize extensively in their contact area, western and central Siberia. This fact can be explained by recent introgression of mtDNA between divergent forms. Recent hybridization might have introduced mtDNA from one species to the other, and that mitochondrial clade might have become fixed in both species (Irwin et al., 2009).

The ortolan bunting (*Emberiza hortulana*) is a passerine bird. It is observed in Europe and western Asia and migrates to tropical Africa. The cytochrome-b sequences are considerably similar in the sister pairs *Emberiza hortulana* and *Emberiza caesia* than in other sister species. This similarity is also remarkable because these species are morphologically distinct. These species are not known to hybridize, however past hybridization leading to introgression could be a possibility (Alstrom et al., 2008).

The crested lark (*Galerida cristata)* is found in Europe and Asia from Portugal to China and in Africa. This species has one of the highest numbers of subspecies among birds with 30 to 60 different subspecies. Genetic divergence among subspecies is small with some exceptions. Subspecies are mainly classified according to plumage color, bill size and bill shape (Guillaumet et al., 2006).

The European bee-eater (*Merops apiaster*) breeds throughout Europe, North Africa and western Asia. It also shows very low levels of intraspecific genetic diversity (Marks et al., 2007) and does not have any recognized subspecies.

The black-eared wheatear (*Oenanthe hispanica*) and the whinchat (*Saxicola rubetra)* are small migratory passerine birds which were previously classified in the family Turdidae, but they now are classified in Muscicapidae. Genetic studies indicate that *Oenanthe hispanica* and *Oenanthe pleschanka* are sister species. Aliabadian et al. (2007) argued that the main reason of their genetic similarity is their ability to hybridize in their contact zones.

The golden oriole or European golden oriole (*Oriolus oriolus*) is a passerine bird which breeds in Europe and western Asia and winters in South Africa. It is divided into two subspecies, *Oriolus oriolus oriolus* and *Oriolus oriolus kundoo*. The latter one is also accepted as a new species *Oriolus kundoo* (Jonsson et al., 2010).

The house sparrow (*Passer domesticus*) and the Eurasian tree sparrow (*Passer montanus*) are passerine birds. They breed naturally in most of Europe, the Mediterranean region, and Asia. *Passer domesticus* is also introduced to many parts of the world like America and Australia. The origin of *Passer* genus seems to be African because the highest number of extant species is on this continent (Allende et al., 2001).

The blackcap (*Sylvia atricapilla)* is a warbler with a wide distribution from the Atlantic Islands in the west to the Caucasus. Although the blackcap has five subspecies, genetic studies show that there is no genetic divergence among subspecies (Dietzen et al., 2008). The barred warbler (*Sylvia nisoria*) is also a widespread warbler that breeds throughout Eastern Europe and temperate Asia.

Finally, the common blackbird (*Turdus merula*) breeds in Europe, Asia, and North Africa. Common blackbird may be resident or migratory according to its latitude. It was recently suggested that the several subspecies of *Turdus merula* could be considered as distinct species based on divergences in song or plumage (Voelker et al., 2007).

1.5. Thesis Objective

Main objective of this study is to utilize the DNA barcoding technique for the first time for birds in Turkey. This approach will help the determination of genetic diversity of bird species in the Kars-Iğdır area and make it possible to compare this diversity to COI barcodes of these species from different parts of the world. This comprises a first step in preparing a database of genetic diversity for the species in the region. Determination of genetic diversity is also important in terms of determining species stability and help in their conservation. Also, genetically diverse variants of morphologically known species are evaluated, which will aid in potential discovery of new species.

2. MATERIALS AND METHODS

2.1. Field Methods

Blood samples are collected from Iğdır, Aras ringing station. Aras station's coordinates are 40º07'16"N and 43º35'00" E. Station is located in the north part of Aras River. Birds are caught, in collaboration with KuzeyDoğa Society with very thin non-harmful nets. According to blood sample collecting protocols, vaseline is used to make bird's brachial vein visible. Then, the vein is punctured with a 27 or 30 gauge needle and blood collected directly into 50 microliter glass tubes with a suction device. It is then transferred to Eppendorf tubes, which contain Longmire buffer. Finally, the region used for blood collection is sterilized with alcohol. Blood samples are kept in cold. The amount of blood that can be safely collected from a bird is 1% of its body weight. So, even for a bird weighing 100 grams, 1000 microliters of blood can be collected safely, so the collected sample is much lower than allowable limits. Blood sample collection was done between May 2009 and October 2009.

2.2. Laboratory Methods

2.2.1. DNA Extraction

For the DNA extraction of bird blood samples, Roche Blood Kit and Invitrogen DNA extraction Kit were used and the manufacturer protocols were applied. The isolated DNA was checked on 1% agarose gels, prepared in 1X TBE (Tris base, boric acid, EDTA) buffer with ethidium bromide. For each reaction, 2 µl of the isolated DNA was loaded on the gel, mixed with 2 µl of 2X loading dye (Fermantas). Samples were run at 90 V for 30 minutes and finally, the band images were taken under ultraviolet light by a Biorad Gel Doc Imaging System (Figure 2.1).

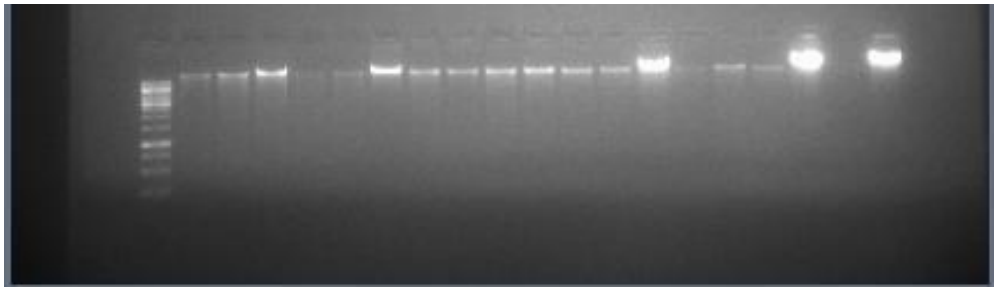

Figure 2.1. The agarose gel photograph of DNA samples after electrophoresis at 90 V for 30 minutes.

2.2.2. PCR Amplification and PCR Product Purification

COI is used as the standard molecular marker of choice. COI gene is the unique DNA marker for the Consortium for the Barcode of Life (CBOL) and the All Birds Barcoding Initiative (ABBI) (Nyari et al., 2007). Protocols and primers for COI amplification are followed as in Herbert et al (2004).

For each PCR, 2 µl of DNA was added to a 48 µl reaction mixture. The mixture was composed of 4.5 µl of 10x high fidelity buffer, 4 µl of $MgCl_2$ (25 mM), and 1.5 µl of 10 mM deoxyribonucleotide triphosphate (dNTPs), 2.5 µl of each primer (20 µM), 0.3 µl Taq DNA polymerase and 32.7 µl H_2O. Cycling parameters consisted of an initial denaturation step of 1 min at 94°C followed by 35 cycles of 1 min at 95°C, 1.5 min at 51°C and 1.5 min at 72°C with a final extension step 5 min at 72°C. The PCR products were run in 1 % agarose gel in order to observe the quality of PCR (Figure 2.2).

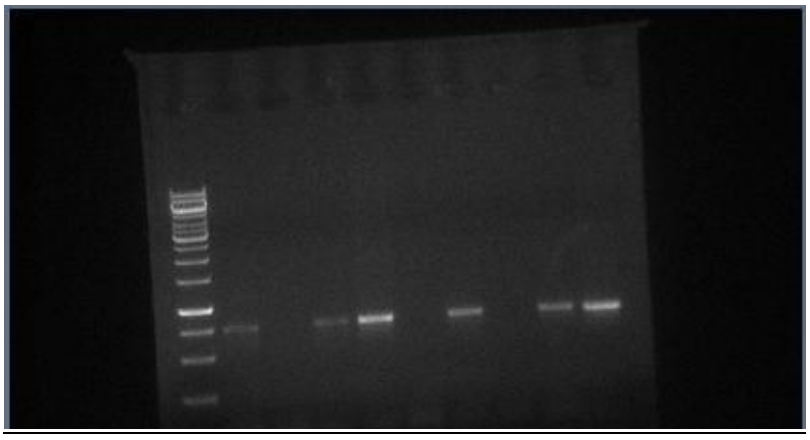

Figure 2.2. The agarose gel photograph of PCR products after electrophoresis at 90 V for 50 minutes.

PCR products of desired length were first compared with DNA ladder, and subsequently were cleaned up for further use in the sequencing reaction by using Roche kits. After clean-up, PCR reaction products were sent to the Macrogen Inc. in South Korea for base sequencing. Obtained base sequences were cleaned with the software Sequencher v. 4.1 (Gene Codes Corp.) program and Clustal X (Thompson et al., 2007) program was used for the sequence alignments. All obtained sequences will be submitted to the Consortium for the Barcode of Life database (BOLD).

2.3. Analytical Method

Phylogenetic trees indicate the evolutionary relationships between species that are believed to come from a common ancestor. Neighbor joining is a phylogeny construction algorithm that clusters taxa according to estimated pairwise evolutionary distances (Sheneman et al., 2006). Main aim of the neighbor-joining method is to find neighbor pairs that minimize the total length of branches (Saitou et al., 1986). A neighbor joining tree for each species was prepared with Iğdır samples combined with sequences downloaded from BOLD, using Kimura 2-parameter distances. All available sequences in BOLD were used for ingroups whereas outgroups were selected from same genus if they are available. Also a tree from all obtained sequences from all species for global comparison was prepared using the software MEGA 4.0 (Tamura et al., 2007). TCS 1.21 (Clement et al., 2000) was used to prepare haplotype networks. Intraspecific and interspecific distances are calculated by MEGA 4.0 using Kimura 2-parameter distances.

3. RESULTS

Seventy three COI sequences from 33 different species are newly generated for this study. 301 samples are added from BOLD. These 33 species belong to 26 different genera. The mean intraspecific divergence was 0.62%. However, in five cases (*Sylvia curruca* 3.2%, *Saxicola maurus* 2.8%, *Phoenicurus phoenicurus* 2.6%, *Parus major* 1.7%, *Caprimulgus europaeus* 1.7%) higher intraspecific divergence, were observed. When these clades are removed, the mean intraspecific divergence is 0.3%. The minimum interspecific distance was 6.8% (Fig. 3.1).

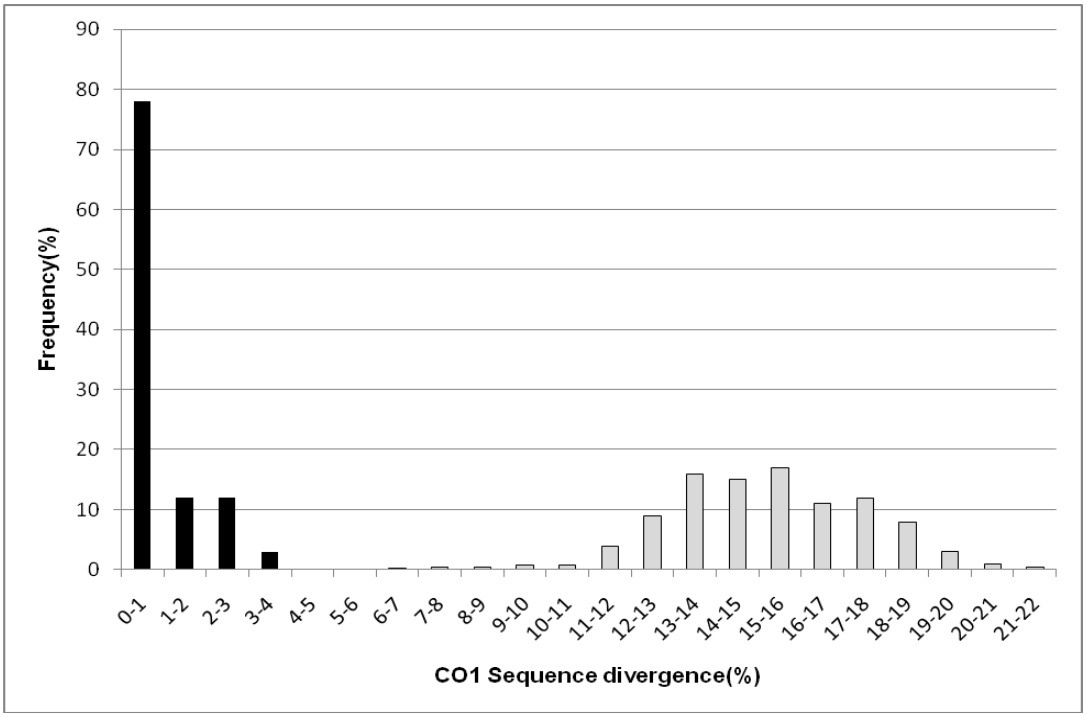

Figure 3.1. Frequency distribution of mean divergences for COI sequences (Kimura 2-parameter model) for 73 samples. Two taxonomic levels are represented: species (dark bars) and genus (gray bars).

A phylogenetic tree is prepared with all sequences used in this study to see the consistency of our results with the current taxonomy (Fig. 3.2).

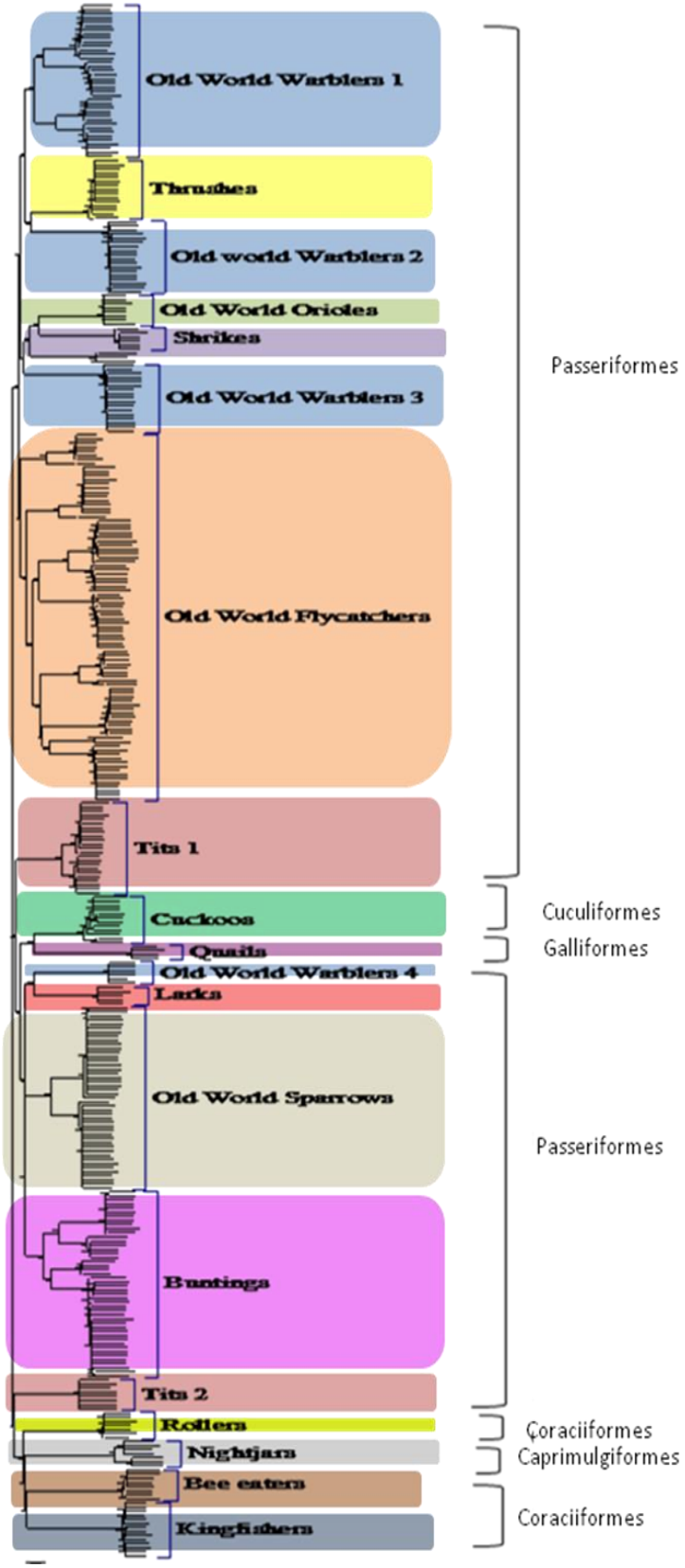

Figure 3.2. Neighbor-joining tree for COI sequences from 374 bird samples.

Clades in Figure 3.2 are highlighted by different colors and contain several species that were analyzed in this study, which is shown in Table 3.1.

Table 3.1. Names of species found in different clades.

Old World Warblers 1	*Sylvia atricapilla, Sylvia nisoria, Sylvia curruca*
Thrushes	*Turdus merula*
Old World Warblers 2	*Acrocephalus palustris*
Old World Orioles	*Oriolus oriolus*
Shrikes	*Lanius minor*
Old World Warblers 3	*Locustella lusciniodes, Phylloscopus trochilus*
Old World Flycatchers	*Ficedula parva, Erithacus rubecula, Phoenicurus phoenicurus, Luscinia svecica, Saxicola maura, Muscicapa striata, Oenanthe hispanica, Saxicola rubetra*
Tits 1	*Parus major*
Cuckoos	*Cuculus canorus*
Quails	*Coturnix coturnix*
Old World Warblers 4	*Cettia cetti*
Larks	*Galerida cristata*
Old World Sparrows	*Passer domesticus, Passer montanus*
Buntings	*Emberiza citrinella, Emberiza hortulana, Emberiza calandra, Emberiza schoeniculus*
Tits 2	*Remiz pendulinus*
Rollers	*Coracias garrulus*
Nightjars	*Caprimulgus europaeus*
Bee Eaters	*Merops apiaster*
Kingfishers	*Alcedo atthis*

Below, a phylogenetic tree with two outgroups is presented for each genus. For each species a map that shows the locations of samples, a phylogenetic tree for that species, and a haplotype network is also prepared. In the haplotype network different

colors which are used to indicate different countries are shown in Table 4.2. It should be noted that in the text below Turkey specifically refers to samples from Kars-Iğdır, analyzed in this study.

Table 3.2. Colors that used to indicate different countries in haplotype networks.

Country	Color
Sweden	Blue
Norway	Red
Russia	Green
Turkey	Purple
Mongolia	Yellow
South Korea	Gray
Kazakhstan	Orange
Argentina	Brown
Canada	Dark Blue
Lithuania	Dark red
England	Dark Green

3.1. *Coturnix coturnix* (Linnaeus, 1758)

Four different samples from three different countries (Russia, Sweden and Turkey) are analyzed for *Coturnix coturnix* (Fig. 3.3). One of the analyzed samples is from Turkey.

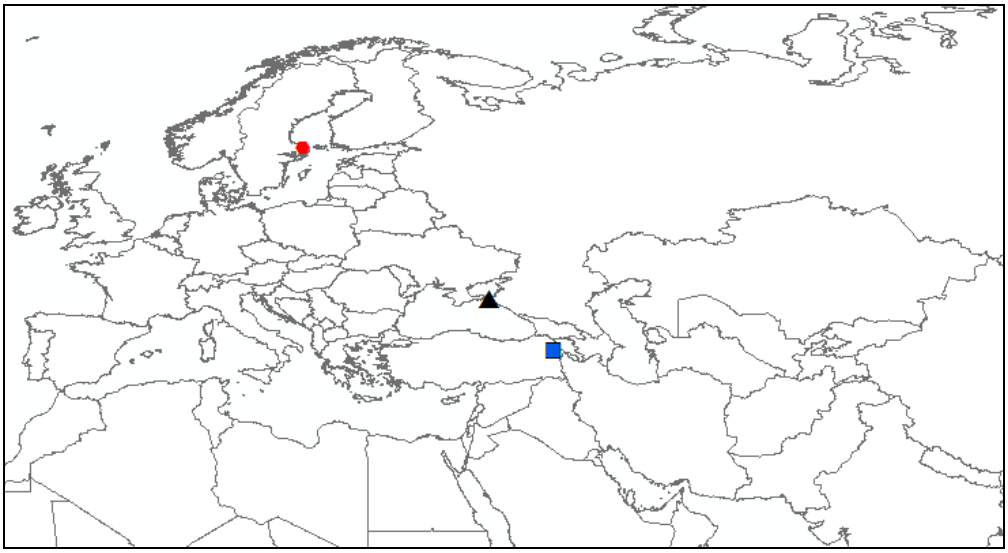

Figure 3.3. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first closest match to Iğdır samples is *Coturnix coturnix* with 100% and second is the same species with 99.59%. Third closest match is from another species *Coturnix japonica* with 97.94% similarity. Neighbor-joining tree contains ten samples from two different species and two outgroups *Phasianus colchicus* and *Perdix perdix* (Fig. 3.4). *Coturnix coturnix* is a monophyletic group sister to *Coturnix japonica*. As observed from haplotype network, all four samples have the same COI sequence (Fig. 3.5).

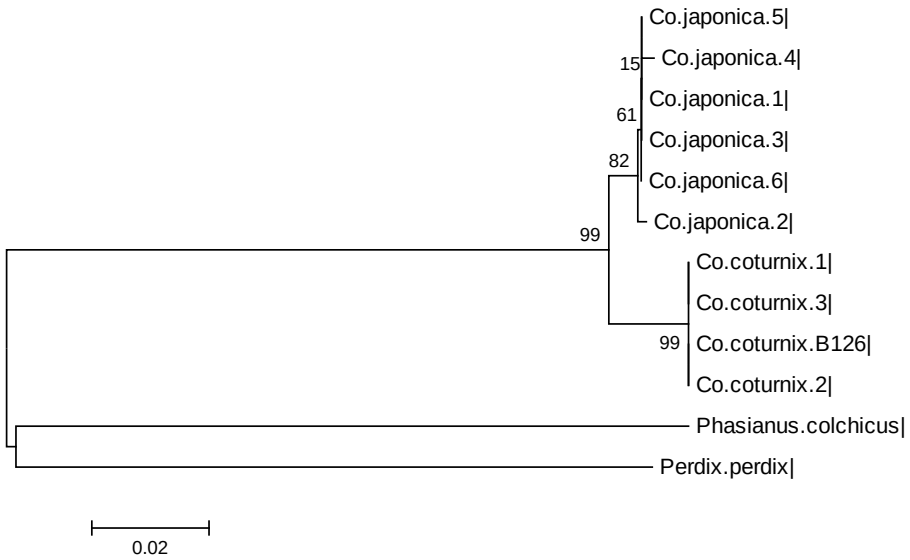

Figure 3.4. Neighbor-joining tree for *Coturnix coturnix.*

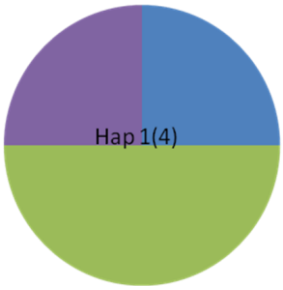

Figure 3.5. Haplotype network for *Coturnix coturnix*.

3.2. *Cuculus canorus* (Linnaeus, 1758)

Twelve different samples from seven countries (Russia, Turkey, Kazakhstan, Mongolia, Sweden, South Korea and Norway) are analyzed for this species. There is one sample collected from Iğdır (Fig. 3.6).

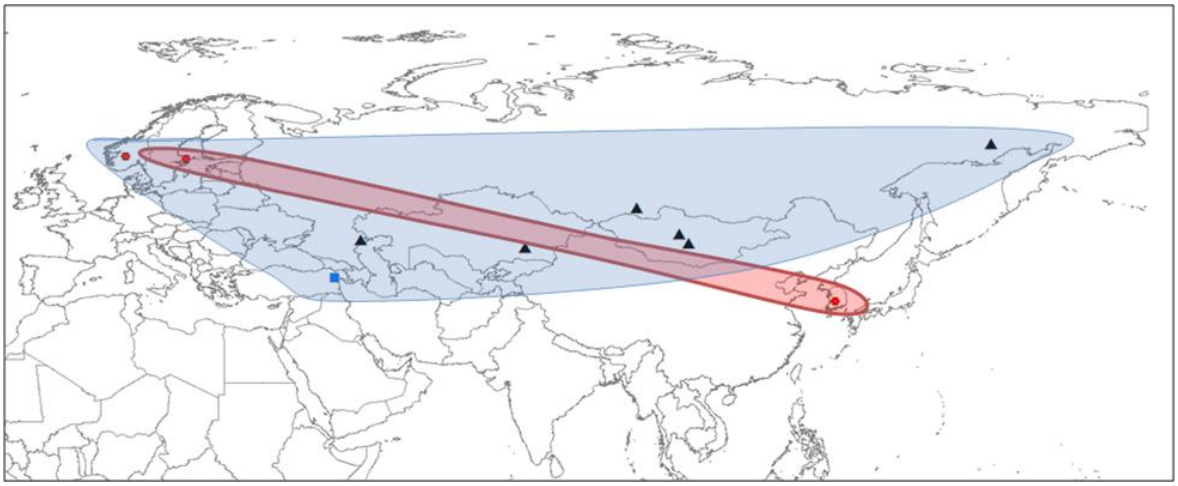

Figure 3.6. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır. Hap 2 and Hap 3 is indicated by red areas and remaining haplotypes are indicated with blue areas.

A comparison with the BOLD database shows first closest match to Iğdır samples is *Cuculus canorus* with 100%. This species has % 99.53 similarities with both *Cuculus canorus* and *Cuculus optatus*. Neighbor-joining tree contains 17 samples from two different species and two outgroups *Coccyzus erythropthalmus* and *Crotophaga ani* (Fig. 3.7). *Cuculus canorus* and *Cuculus optatus* are sister species and they are located closely

with each other in the neighbor-joining tree. This figure also shows that the classification of *Cuculus canorus* and *Cuculus optatus* is not very clear, as can be seen in the paraphyletic distributions in the tree. However, it should be noted that our sample from Iğdır, B128 clustered with other individuals of *Cuculus canorus*.

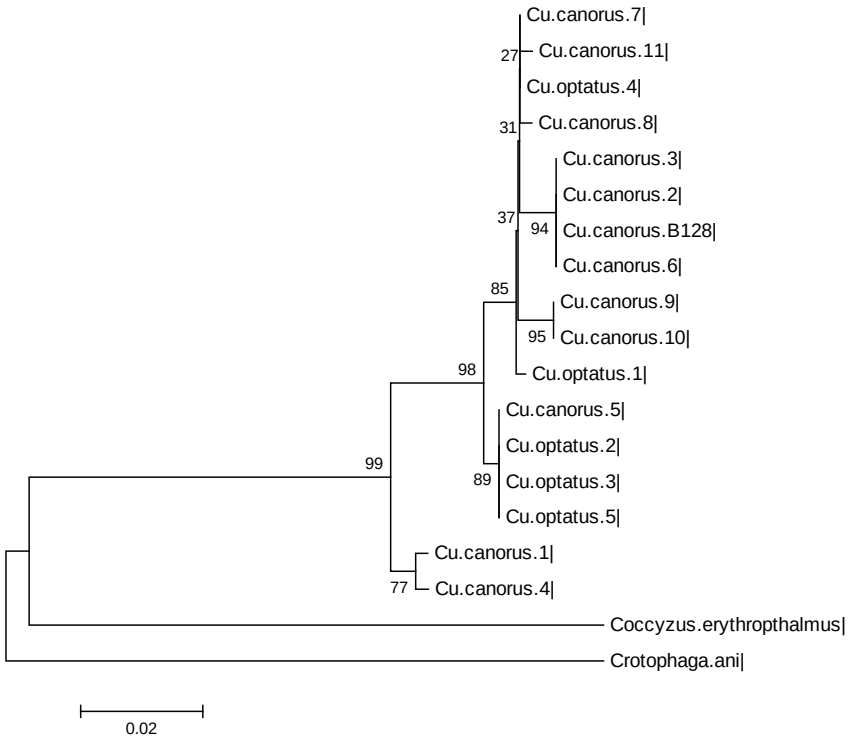

Figure 3.7. Neighbor-joining tree for *Cuculus canorus*.

Eight haplotypes are found in *Cuculus canorus* (Fig. 3.8). Hap 1 is the most common haplotype observed in Iğdır, Russia and Norway. Hap 7 is also observed in more than one country, Russia and Kazakhstan. All other six haplotypes are observed only in one country. Hap 2 and Hap 3 are differentiated from the other haplotypes by 12 bases, and their geographic distribution is indicated with red areas, and the remaining haplotypes are with blue in Fig. 3.6.

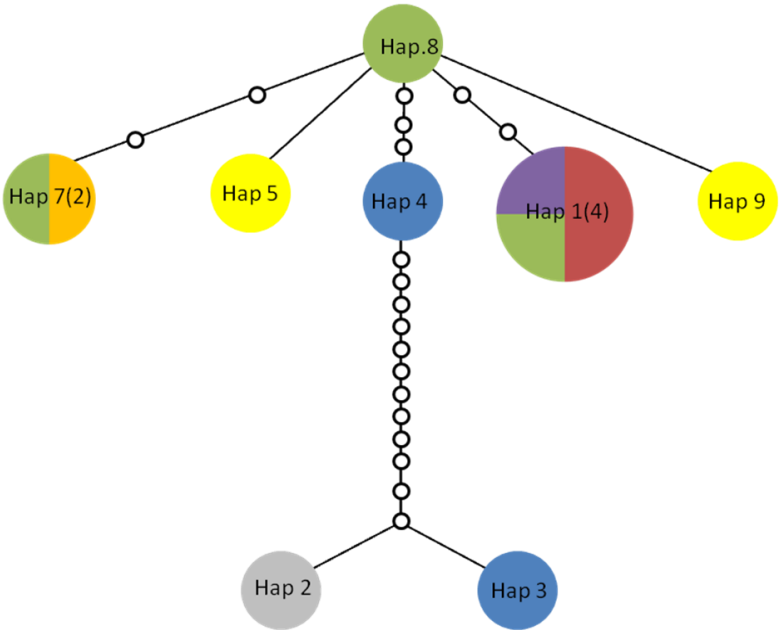

Figure 3.8. Haplotype network for *Cuculus canorus*.

3.3. *Caprimulgus europaeus* (Linnaeus, 1758)

Seven different samples from three different countries (Russia, Turkey, and Norway) are analyzed for *Caprimulgus europaeus* (Fig. 3.9). There is one sample which is collected from Iğdır.

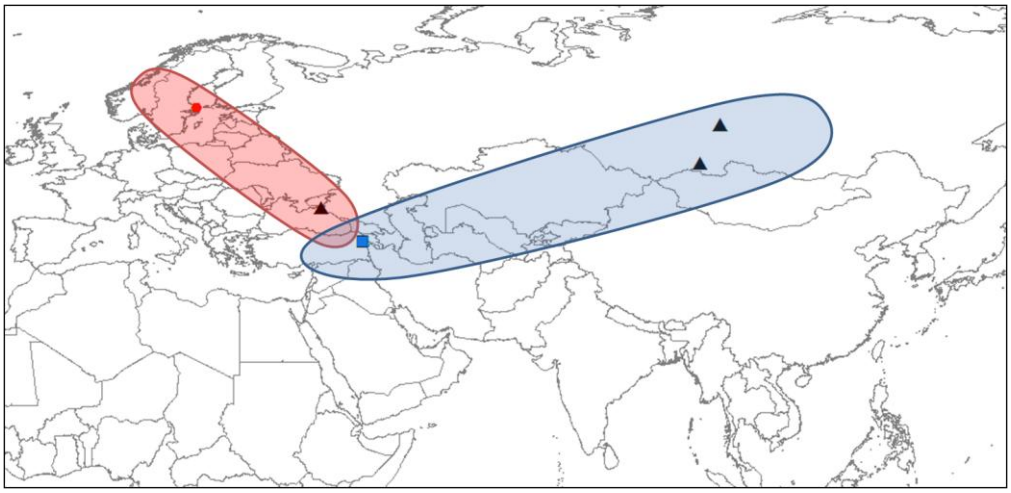

Figure 3.9. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır. Red and blue areas indicate Hap 1 and Hap 2 respectively.

A comparison with the BOLD database shows first and second closest matches to Iğdır samples is *Caprimulgus europaeus* with 100% and 97.08%, respectively. Third closest match is from another species *Caprimulgus aegyptius* with 92.96% similarity. Neighbor-joining tree contains 20 samples from five different species and two outgroups *Nyctidromus albicollis* and *Chordeiles acutipennis* (Fig. 3.10). *C. europaeus* samples form two clades, where the sample that is barcoded from Turkey (B106) falls within one of these groups. These two clades of *C. europaeus* are seen as sister to the *C. indicus* clade.

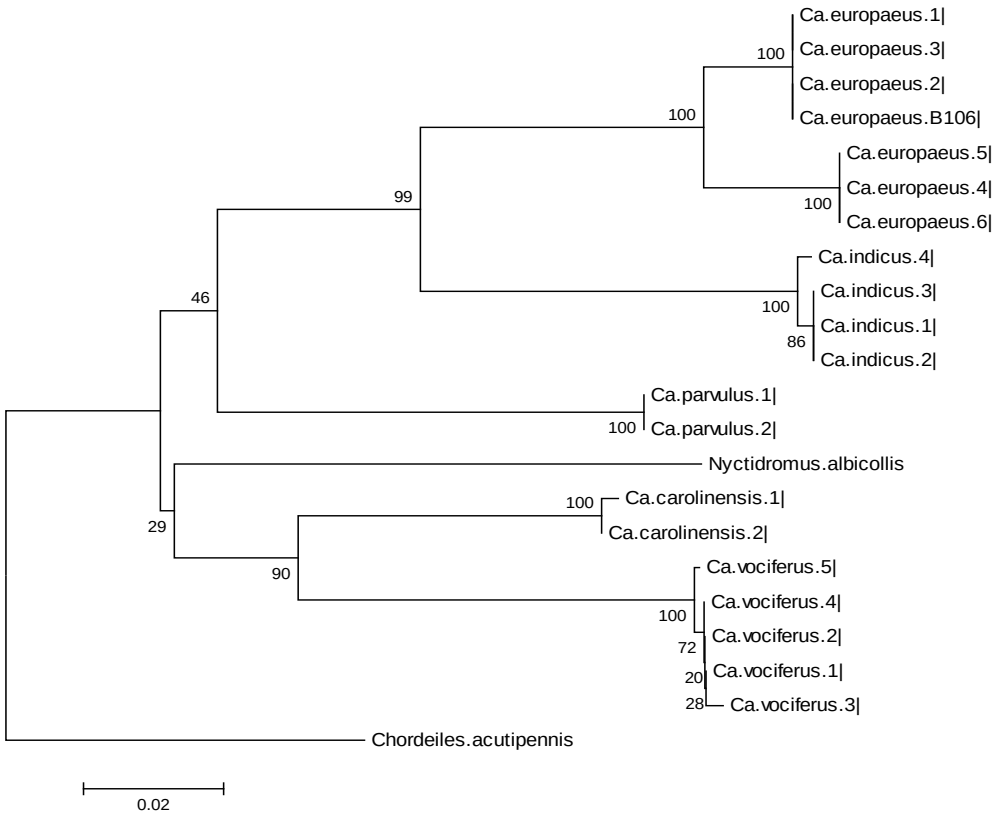

Figure 3.10. Neighbor-joining tree for *Caprimulgus europaeus*.

Two different haplotypes are observed for this species (Fig. 3.11) which is also confirmed by existence of two clades in the map. Hap 1 is observed in Norway and Russia and indicated by red areas whereas Hap 2 is observed in Turkey and Russia and is indicated by blue areas in the map (Fig. 3.9). These two haplotypes are separated by 18 base pairs. As also observed in map, Hap 1 is common in western parts of the species' range whereas Hap 2 is more common in the eastern parts.

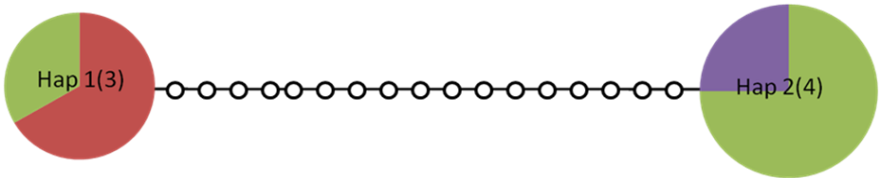

Figure 3.11. Haplotype network for *Caprimulgus europaeus.*

3.4. *Alcedo atthis* (Linnaeus, 1758)

Fourteen different samples from seven different countries (Russia, Turkey, Sweden, Norway, Kazakhstan, Mongolia, and South Korea) are analyzed for this species (Fig. 3.12). There are three samples collected from Iğdır.

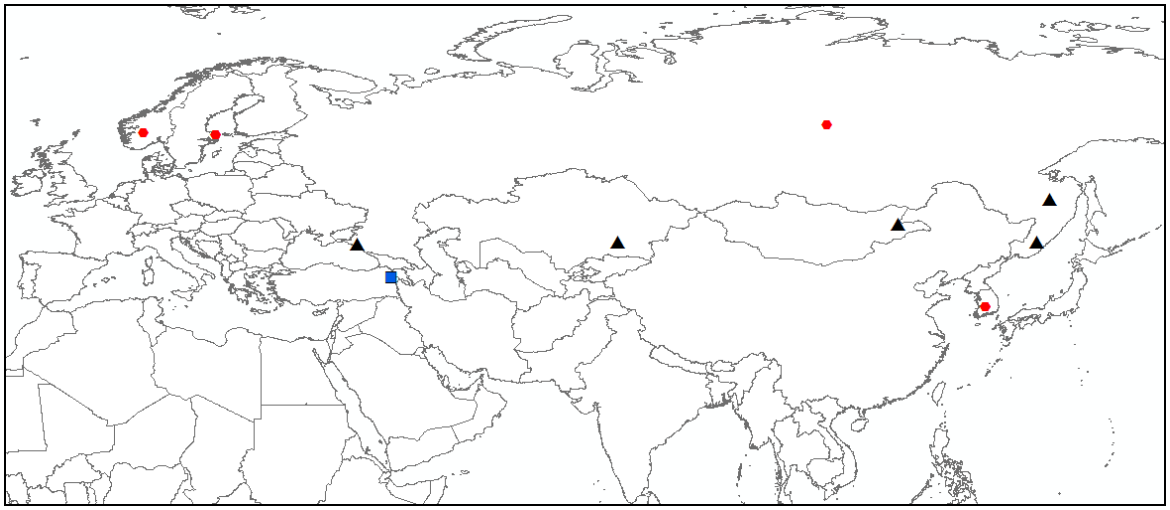

Figure 3.12. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first closest match to Iğdır samples is *Alcedo atthis* with 100%, second with 99.84% and third with 99.69%. There are two outgroups, *Todiramphus sanctus* and *Megaceryle alcyon*, used in the neighbor-joining tree (Fig. 3.13). In the tree the barcoded individuals from Iğdır (B91, B93 and B94) cluster closely with the rest of the barcodes from BOLD, from the entire Palearctic.

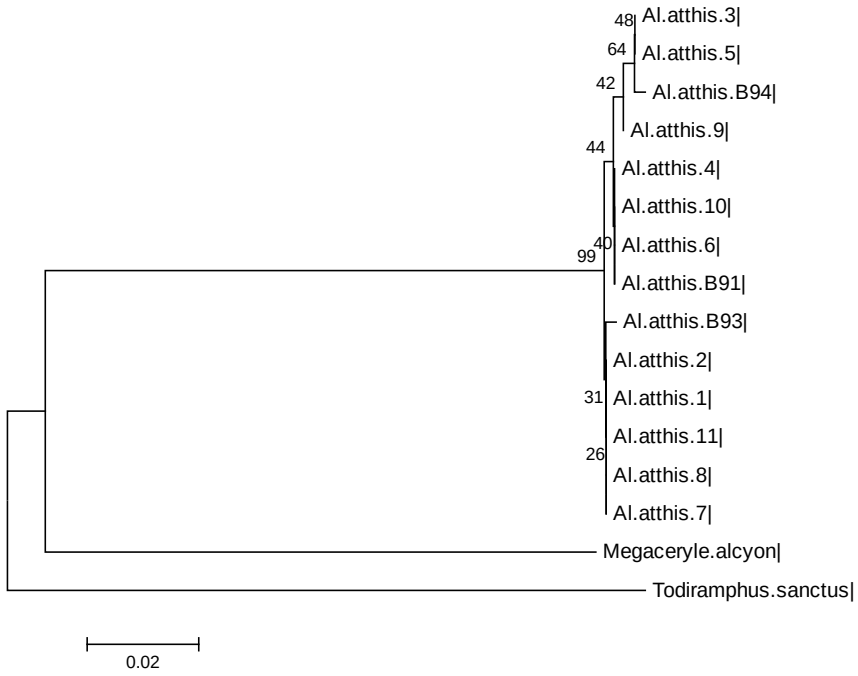

Figure 3.13. Neighbor-joining tree for *Alcedo atthis*.

There are six different observed haplotypes in this species as seen in the haplotype network (Fig. 3.14). Hap 1 and Hap 4 are the most commonly observed haplotypes seen in five different countries Russia, Sweden, Turkey, South Korea and Mongolia. Two new haplotypes (Hap 2 and Hap 6) are recorded from Turkey.

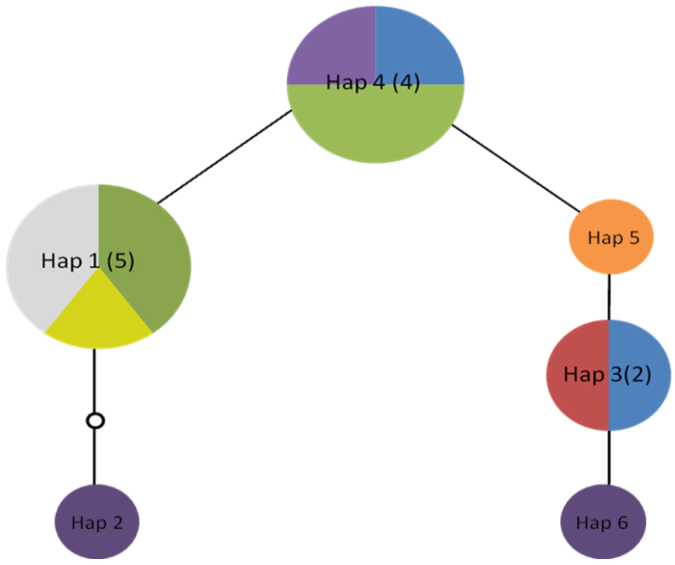

Figure 3.14. Haplotype network for *Alcedo atthis*.

3.5. *Merops apiaster* (Linnaeus, 1758)

Eight samples from two countries (Russia and Turkey) are analyzed for this species (Fig. 3.15). There are three samples which are collected from Iğdır.

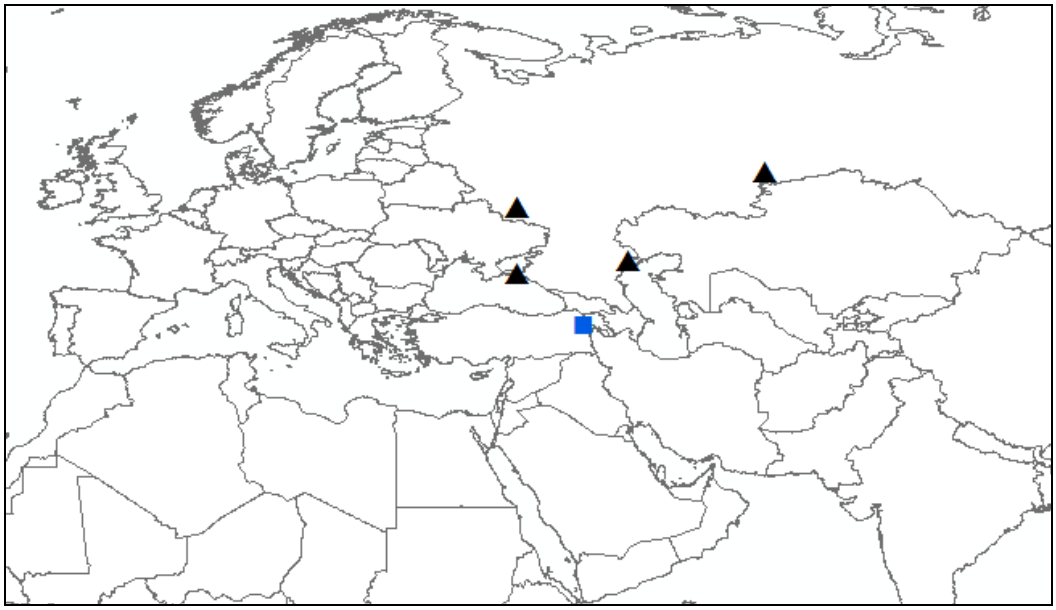

Figure 3.15. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first, second and third closest matches are with *Merops apiaster* with 100%, 99.85% and 99.69% similarity, respectively. Phylogenetic tree contains eight samples from one species (Fig. 3.16). Two outgroups *Coracias garrulus* and *Momotus momota* are used in the neighbor-joining tree. Iğdır samples (B215, B216, and B217) are clustered closely with the rest of the barcodes from BOLD.

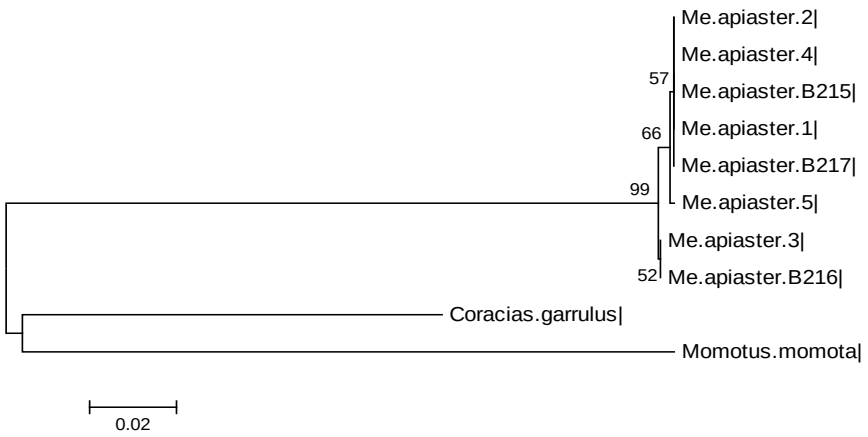

Figure 3.16. Neighbor-joining tree for *Merops apiaster*.

Three different haplotypes are observed for *Merops apiaster*. Hap 1 is observed in five samples and two countries, Turkey and Russia. Hap 2 is also seen in same two countries, but in two samples, and Hap 3 is observed only in Russia (Fig. 3.17).

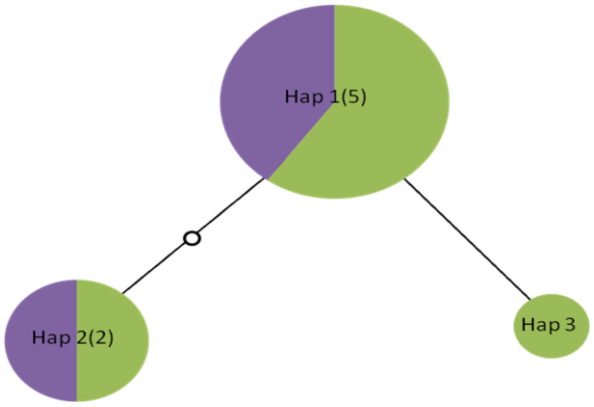

Figure 3.17. Haplotype network for *Merops apiaster*.

3.6. *Coracias garrulus* (Linnaeus, 1758)

Seven different samples from four different countries (Russia, Turkey, Sweden, and Kazakhstan) are analyzed for this species (Fig. 3.18). Two of analyzed samples are collected from Iğdır.

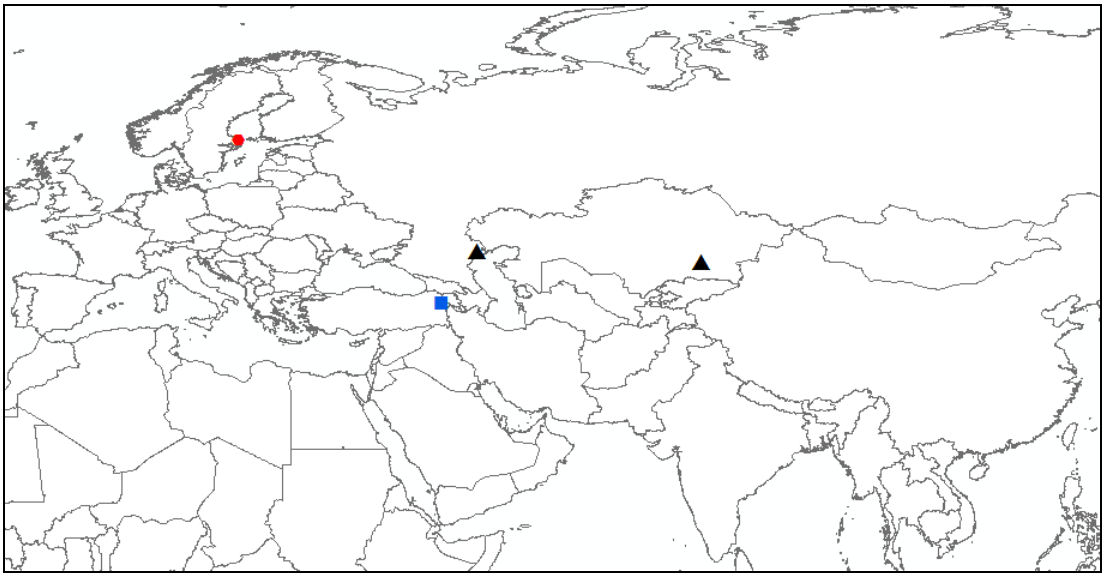

Figure 3.18. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first, second and third closest matches to Iğdır samples is *Coracias garrulus* with 100%, 99.65% and 99.53 %, respectively. Neighbor-joining tree contains seven samples from one species and two outgroups *Eurystomus orientalis* and *Alcedo atthis* (Fig. 3.19). In the tree the barcoded individuals from Iğdır (B127 and B129) cluster closely with the rest of the barcodes from BOLD.

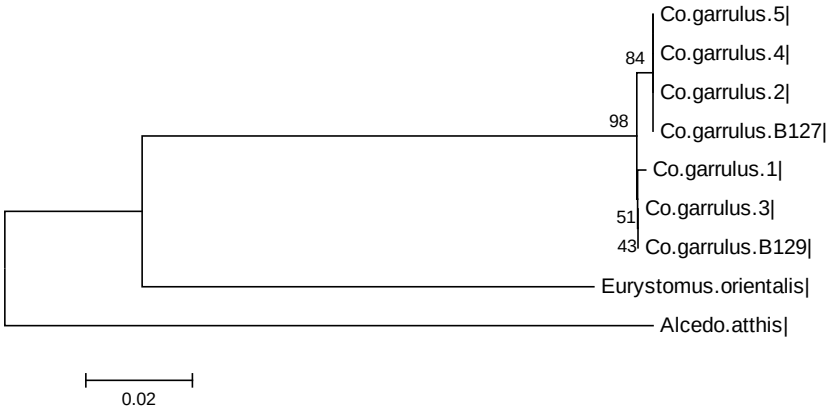

Figure 3.19. Neighbor-joining tree for *Coracias garrulus*.

There are three different observed haplotypes for *Coracias garrulus* (Fig. 3.20). Hap 2 is observed in Turkey and Russia. Hap 3 is most common haplotype and is seen in Turkey, Russia and Kazakhstan, in four samples. Hap 1 is observed only in Sweden.

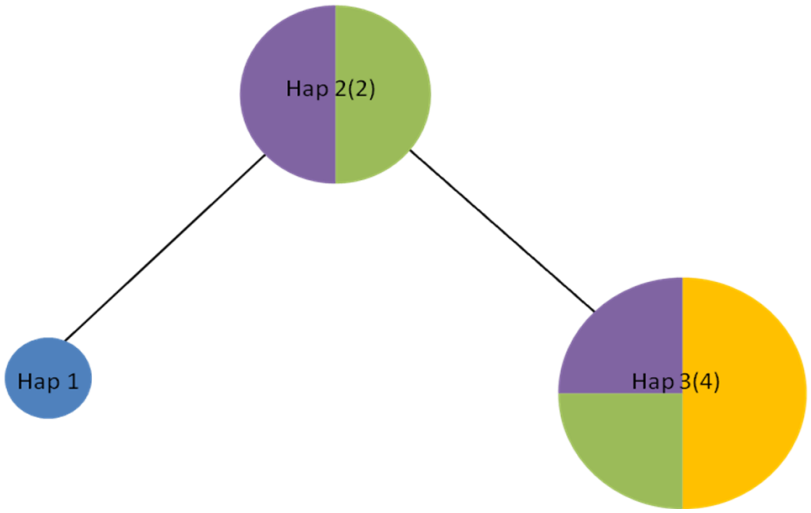

Figure 3.20. Haplotype network for *Coracias garrulus*.

3.7. *Lanius minor* (Gmelin, 1788)

Six samples from two different countries (Russia and Turkey) are analyzed for *Lanius minor*. There is one sample collected from Iğdır (Fig. 3.21).

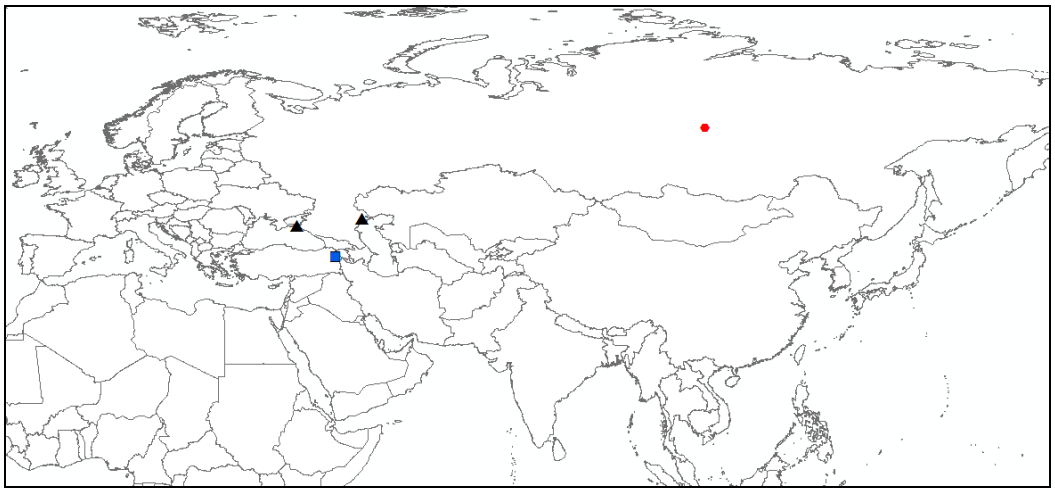

Figure 3.21. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first, second and third closest matches are with *Lanius minor* with 100%, 99.52% and 99.48% similarity, respectively. Phylogenetic tree contains 62 samples from ten species (Fig. 3.22). Two outgroups, *Delichon urbicum* and *Hirundo rustica* are used in the neighbor-joining tree. Iğdır sample (B190) is clustered closely in the tree with the other *L. minor* samples from BOLD.

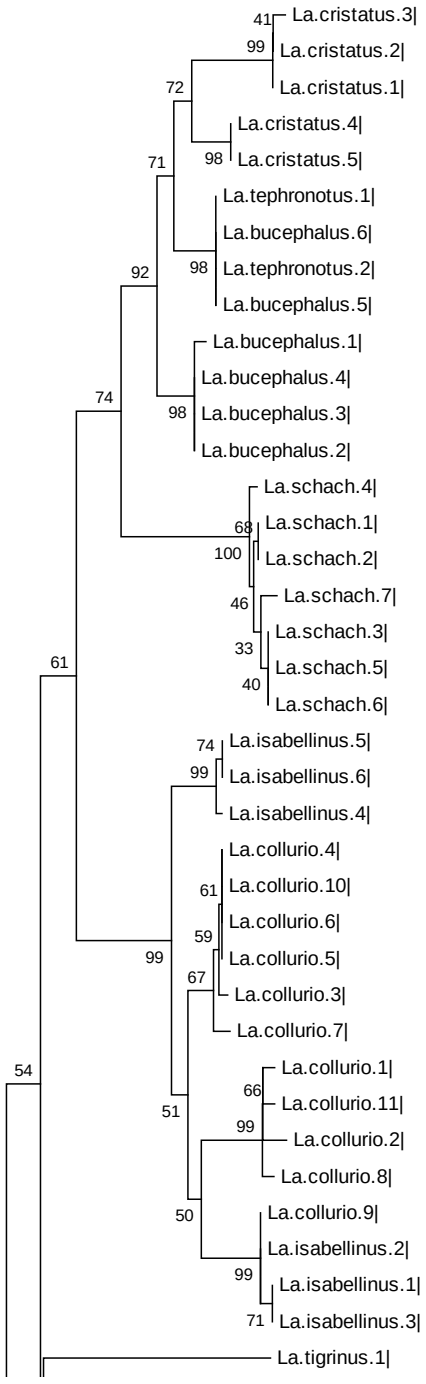

Figure 3.22. Neighbor-joining tree for *Lanius minor*.

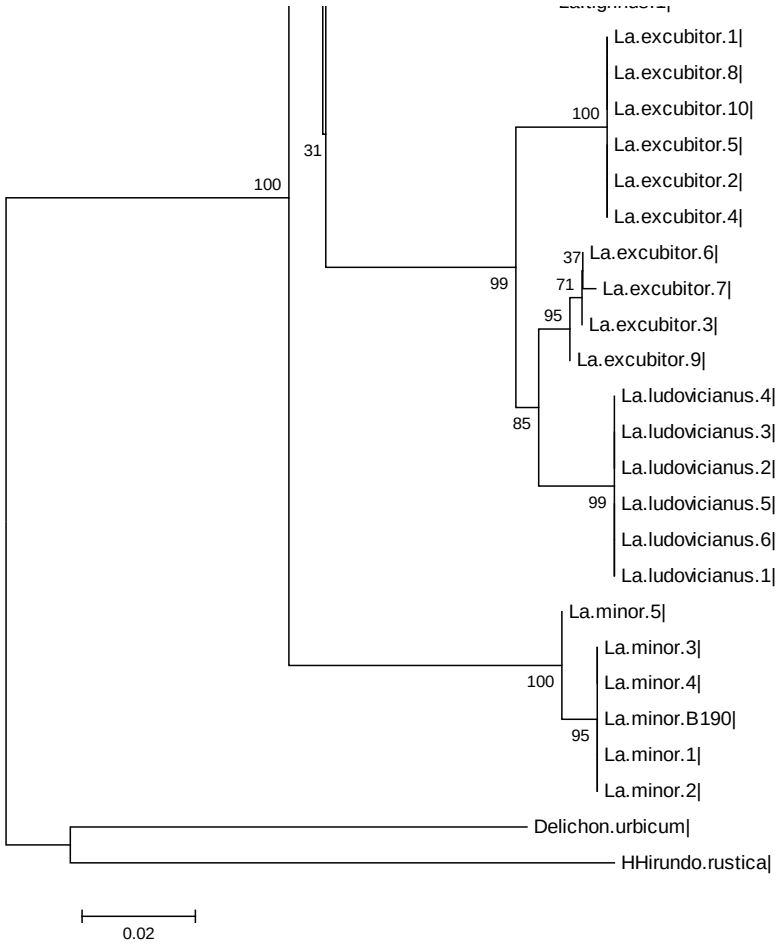

Figure 3.22. continued.

Two different haplotypes with three base pair differences are observed in Russia. Hap 2 is seen both in Turkey and Russia, whereas Hap 1 is observed only in Russia (Fig. 3.23)

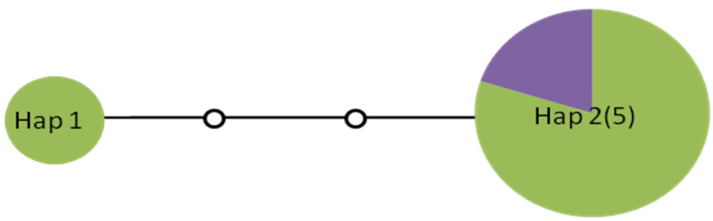

Figure 3.23. Haplotype network for *Lanius minor*.

3.8. *Oriolus oriolus* (Linnaeus, 1758)

Eight different samples from three different countries (Russia, Turkey, and Sweden) are analyzed for this species (Fig. 3.24). There are two samples collected from Iğdır.

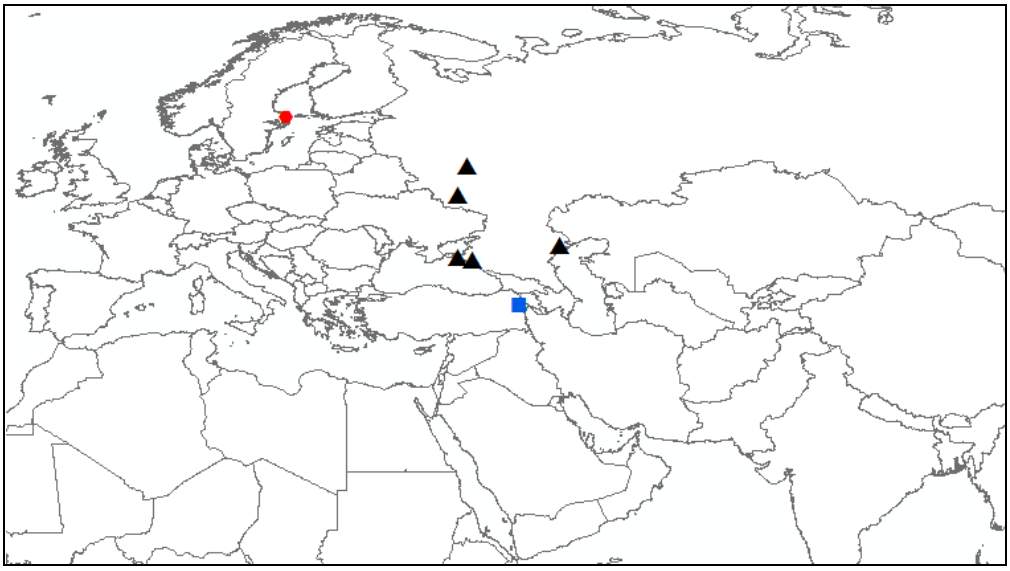

Figure 3.24. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first and second closest matches with Iğdır samples is *Oriolus oriolus* with 100% and 99.84% similarity, respectively. Third closest match is *Oriolus chinensis* with 96.87% similarity. Phylogenetic tree contains 13 samples from two different species (Fig. 3.25). Two outgroups, *Luscinia luscinia* and *Ficedula parva* are used in the neighbor-joining tree. *Oriolus oriolus* formed a monophyletic clade with Iğdır samples (B251, B252) and it is a sister clade of *Oriolus chinensis*.

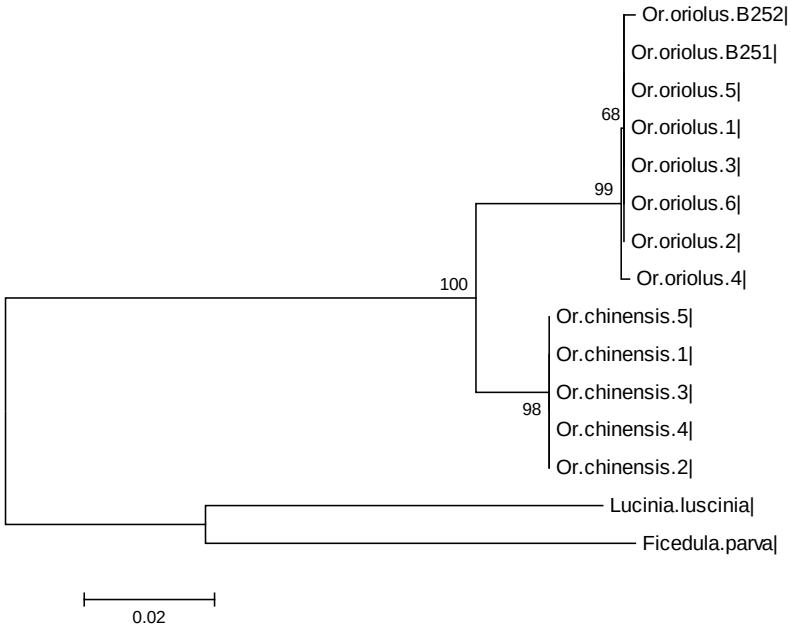

Figure 3.25. Neighbor-joining tree for *Oriolus oriolus*

Three different haplotypes are observed for this species. Hap 2 is observed in six samples in three different countries, Russia, Turkey and Sweden (Fig. 3.26). Hap 3 is a new haplotype observed only in Turkey.

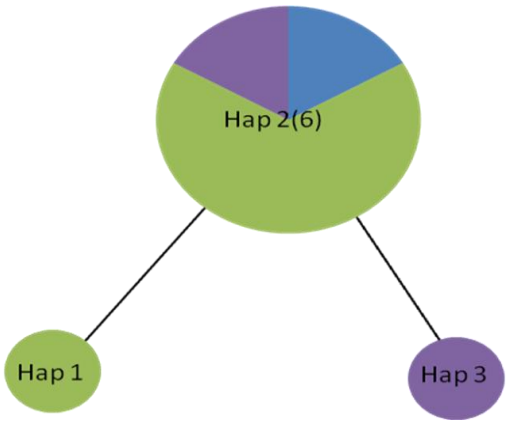

Figure 3.26. Haplotype network for *Oriolus oriolus*.

3.9. *Galerida cristata* (Linnaeus, 1758)

Five samples from three different countries (Russia, Turkey, and Mongolia) are analyzed for *Galerida cristata*. There is one sample which is collected from Iğdır (Fig. 3.27).

Figure 3.27. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first and second closest matches are with 100% and 99.81% similarity, respectively, to *Galerida cristata*. Third closest match is *Alauda arvensis* with 92.29% similarity. Phylogenetic tree contains five samples from one species (Fig. 3.28). Two outgroups, *Alauda arvensis* and *Lullula arborea* are used in the neighbor-joining tree. *Galerida cristata* formed a monophyletic group with two haplotypes differing by one base pair.

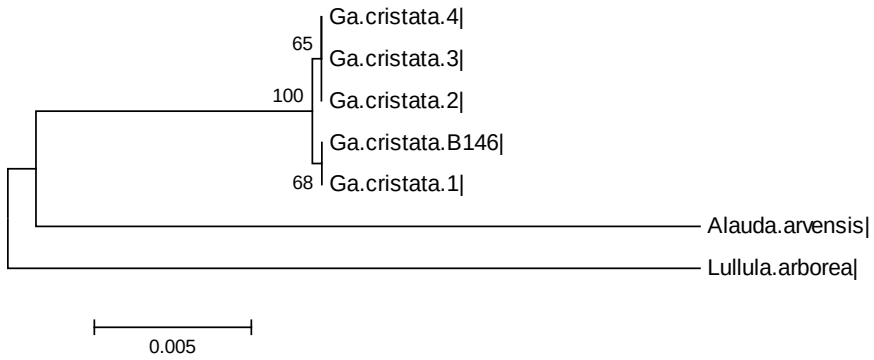

Figure 3.28. Neighbor-joining tree for *Galerida cristata*.

Two different haplotypes are observed for this species. Hap 1 is observed in Russia in three samples, whereas Hap 2 is observed in Turkey and Mongolia (Fig. 3.29).

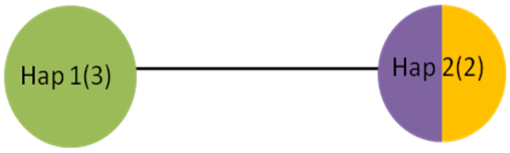

Figure 3.29. Haplotype network for *Galerida cristata*.

3.10. *Parus major* (Linnaeus, 1758)

Twenty three different samples from seven different countries (Russia, Turkey, Lithuania, Norway, Kazakhstan, South Korea, and Sweden) are analyzed for this species Fig. 3.30). Two samples are collected from Iğdır.

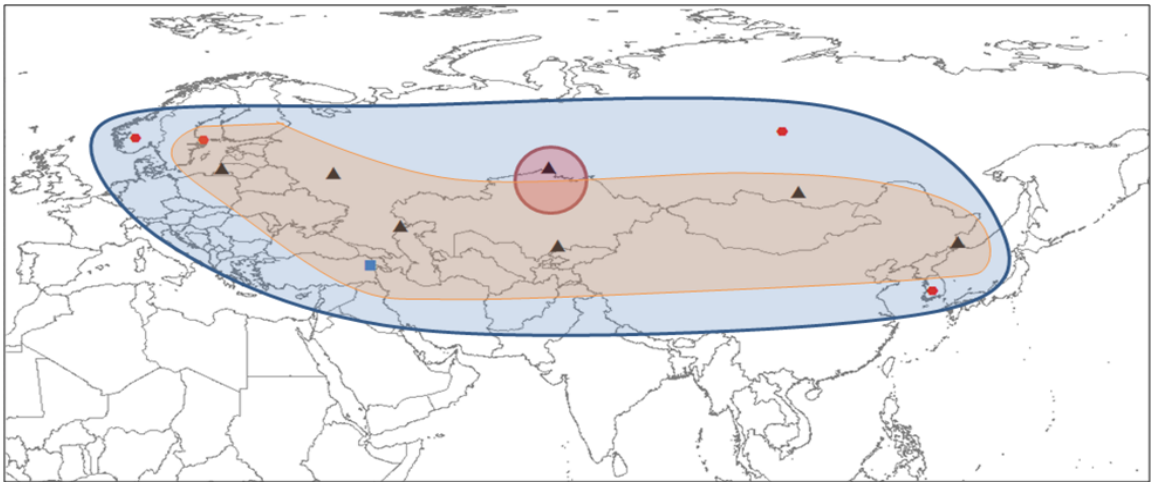

Figure 3.30. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır. Red, orange and blue areas indicate Hap 4; Hap 3, 5; and Hap 1, 2 respectively.

A comparison with the BOLD database shows first, second and third closest matches with Iğdır samples is *Parus major* with 100% , 99.84% and 99.69% similarity, respectively. Phylogenetic tree contains 23 samples from one species (Fig. 3.31). Two outgroups *Baeolophus wollweberi and Poecile atricapillus* are used in the neighbor-joining tree. *Parus major* samples form two clades, where the samples that were barcoded from Turkey (B258 and B259) fell within one of these groups.

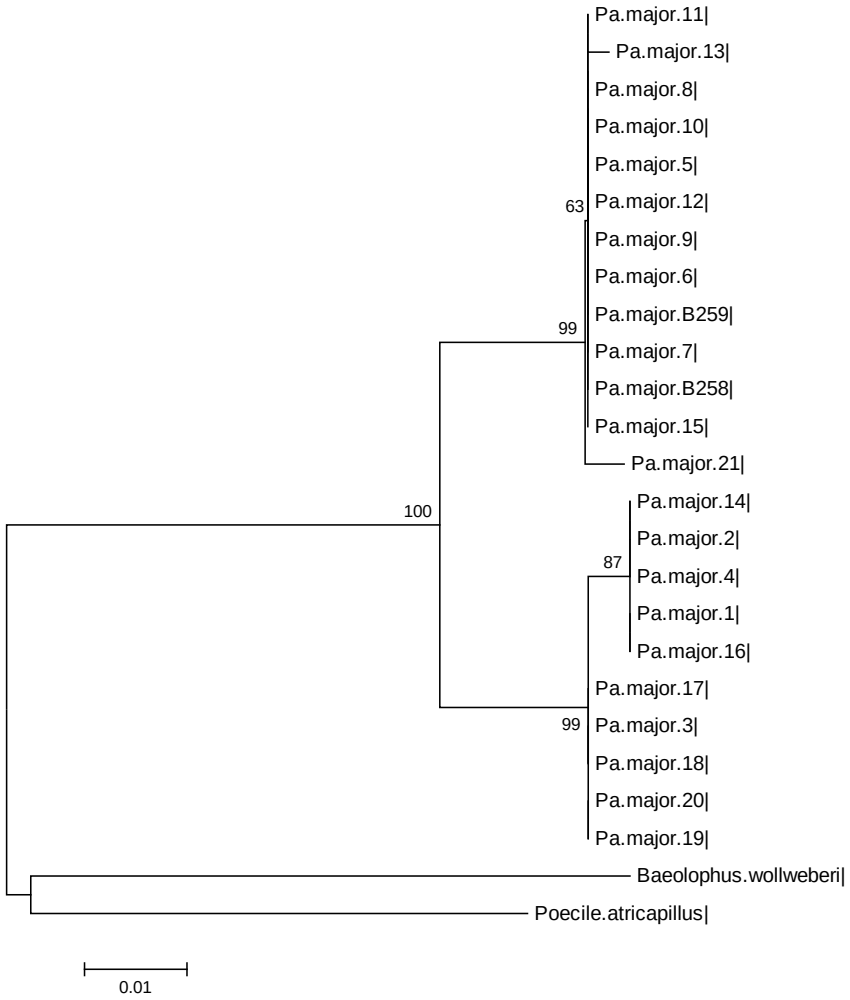

Figure 3.31. Neighbor-joining tree for *Parus major*.

Five different haplotypes are observed for this species. Hap 3 is observed in 11 different samples from five countries (Russia, Iğdır, Sweden, Norway and Lithuania). These are very different (16 base pairs) from Hap 1 and Hap 2, which are observed in three different countries including Russia, South Korea and Norway. Hap 4 is only observed in one sample from Kazakhstan and Hap 5 is only observed in Russia (Fig. 3.32). Two main clades are observed in the neighbor-joining tree because of 16 base pairs difference of Hap 1 and 2 from other haplotypes. The distribution of these haplogroups can be seen in Figure 3.73. Hap 4 is indicated with red, Hap 3 and Hap 5 are indicated with orange, and Hap 1 and Hap 2 are indicated with blue.

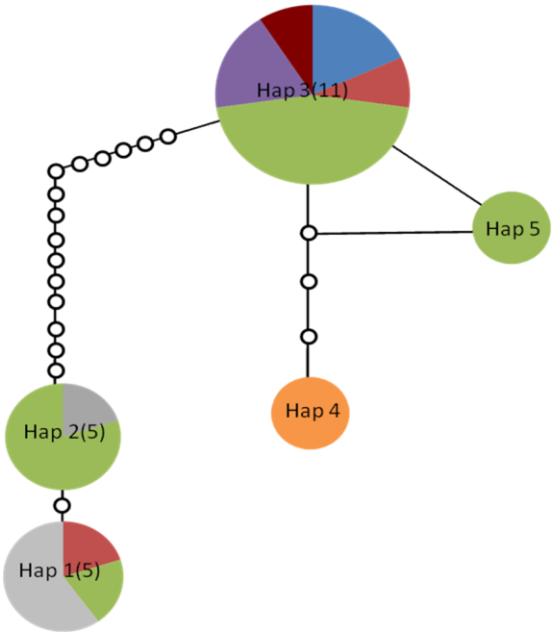

Figure 3.32. Haplotype network for *Parus major*.

3.11. *Remiz pendulinus* (Linnaeus, 1758)

Eight different samples from Sweden and Turkey are analyzed for *Remiz pendulinus*. There are six samples collected from Iğdır (Fig. 3.33).

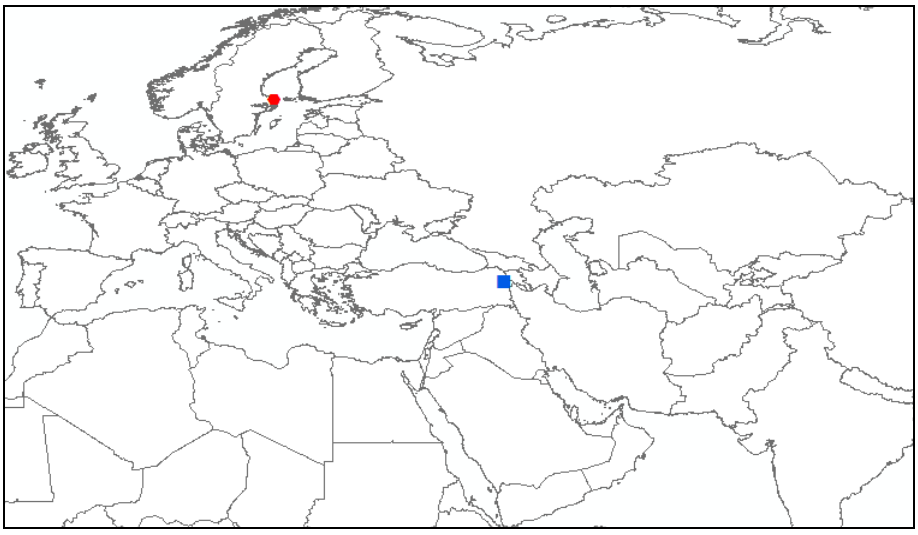

Figure 3.33. The locations for which COI Barcode Data were available from BOLD. The red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first closest match with Iğdır samples is *Remiz pendulinus* with 100% similarity. Second closest match is *Anthoscopus minutus* with 90.2%. Third closest match is *Baeolophus wollweberi* with 89.87% similarity. Phylogenetic tree contains eight samples from one species (Fig. 3.34). Two outgroups *Lanus minor* and *Auriparus flaviceps* are used in the neighbor-joining tree. Haplotype network indicates that both Turkish and Swedish samples are identical (Fig. 3.35).

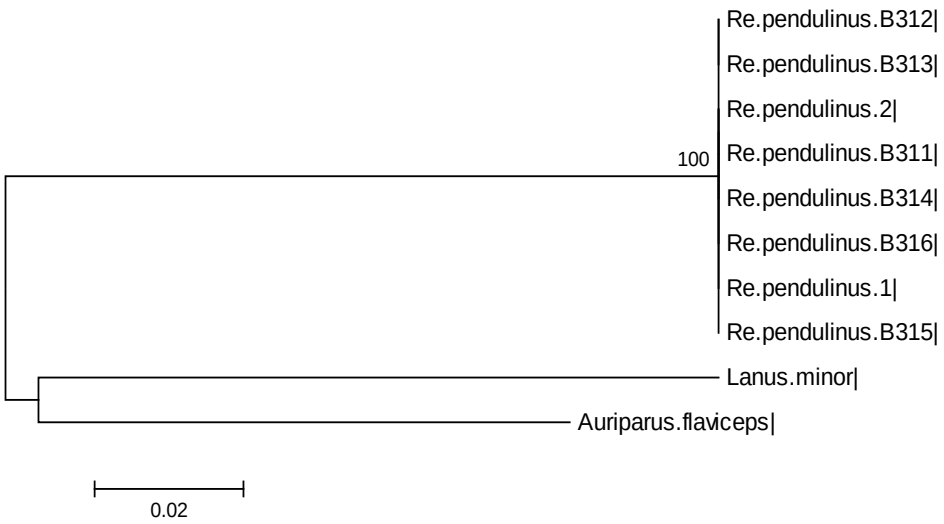

Figure 3.34. Neighbor-joining tree for *Remiz pendulinus*.

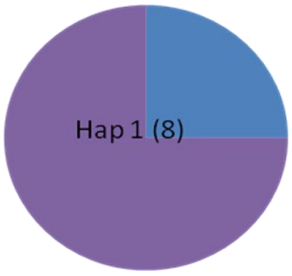

Figure 3.35. Haplotype network for *Remiz pendulinus*.

3.12. *Cettia cetti* (Temminck, 1820)

Six different samples from two different countries (Russia and Turkey) are analyzed for *Cettia cetti* (Fig. 3.36). Five of these six samples are collected from Iğdır.

Figure 3.36. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first closest match to Iğdır samples is *Cettia cetti* with 100%, second is *Carduelis carduelis* with 88.48%, and third closest match is *Paroaria coronata* with 87.48% similarity. Neighbor-joining tree contains 18 samples from two different species and two outgroups *Acrocephalus palustris* and *Bradypterus tacsanowskius* (Fig. 3.37). The tree shows that *Cettia diphone* is sister to *C. cetti* and both the tree and haplotype network indicate that all *Cettia cetti* samples have the same COI sequence (Fig. 3.38).

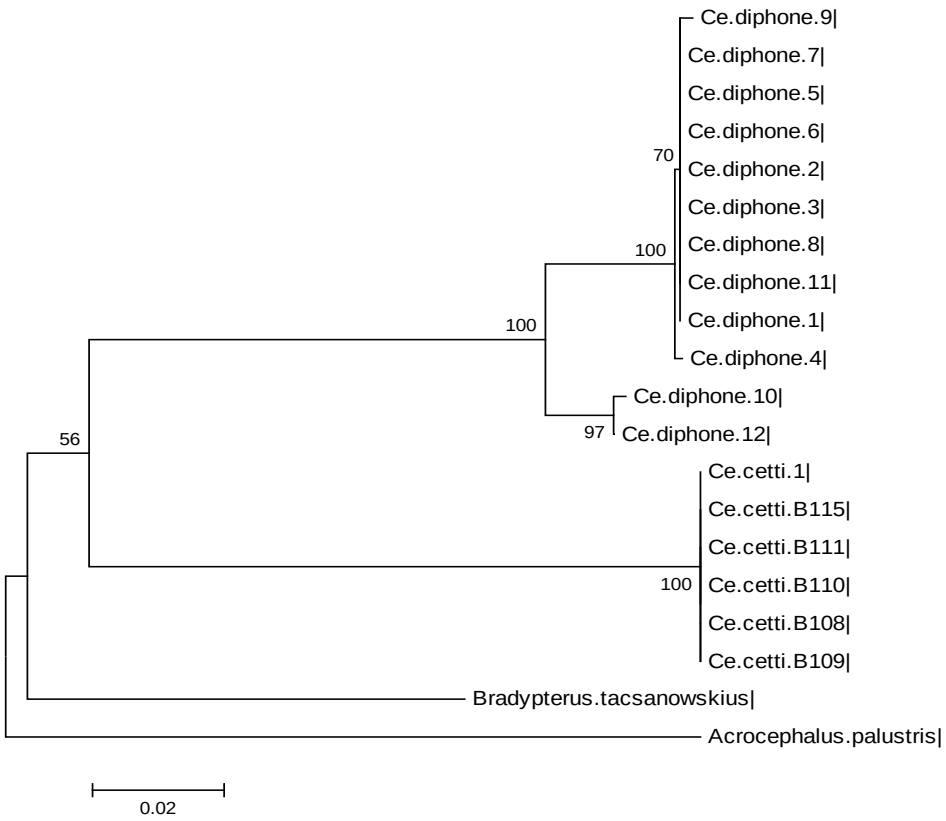

Figure 3.37. Neighbor-joining tree for *Cettia cetti*.

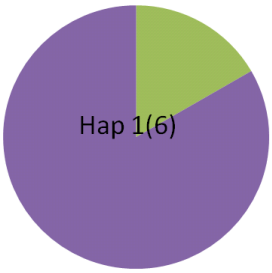

Figure 3.38. Haplotype network for *Cettia cetti*.

3.13. *Phylloscopus trochilus* (Linnaeus, 1758)

Seventeen different samples from four different countries including Russia, Turkey, Norway, and Sweden) are analyzed for this species. There are four samples collected from Iğdır (Fig. 3.39).

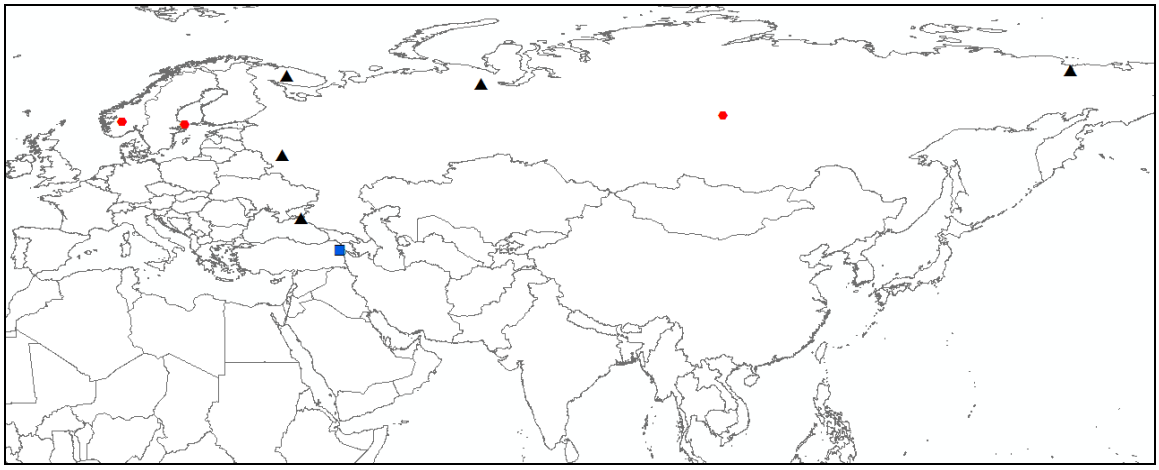

Figure 3.39. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first and second closest matches with Iğdır samples is *Phylloscopus trochilus* with 99.82% and 99.63% similarity, respectively. Third closest match is *Phylloscopus canariensis* with 92.82% similarity. Phylogenetic tree contains 98 samples and 15 species (Fig. 3.40). Two outgroups *Locustella luscinioides and Cettia cetti* are used in the neighbor-joining tree. *Phylloscopus trochilus* samples from Iğdır and BOLD formed a monophyletic group in this tree.

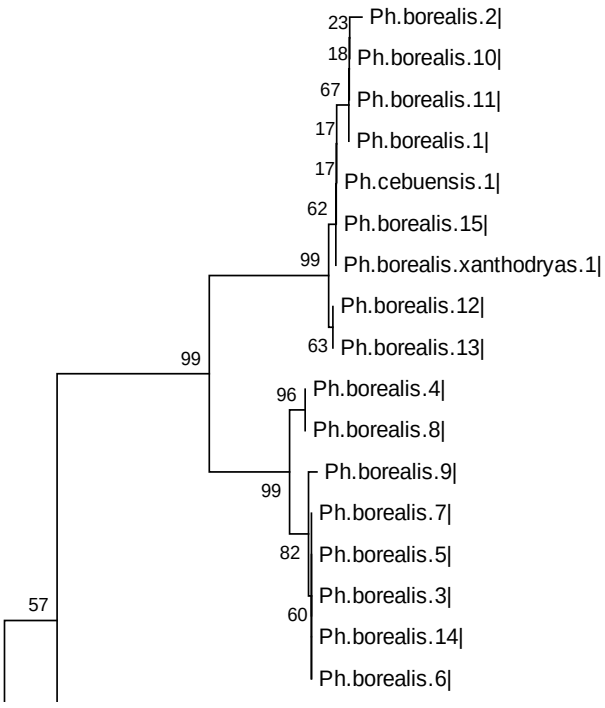

Figure 3.40. Neighbor-joining tree for *Phylloscopus trochilus*.

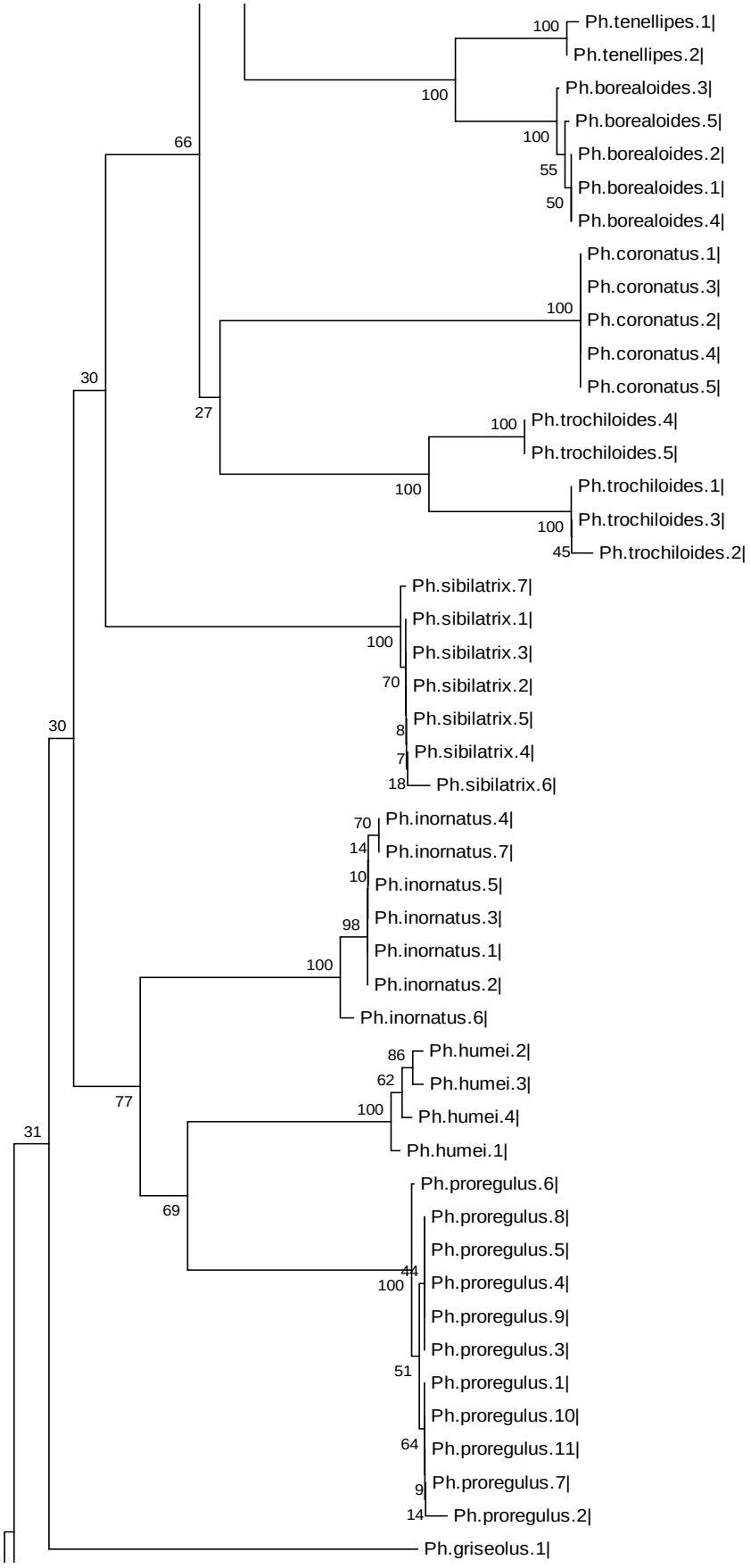

Figure 3.40. continued.

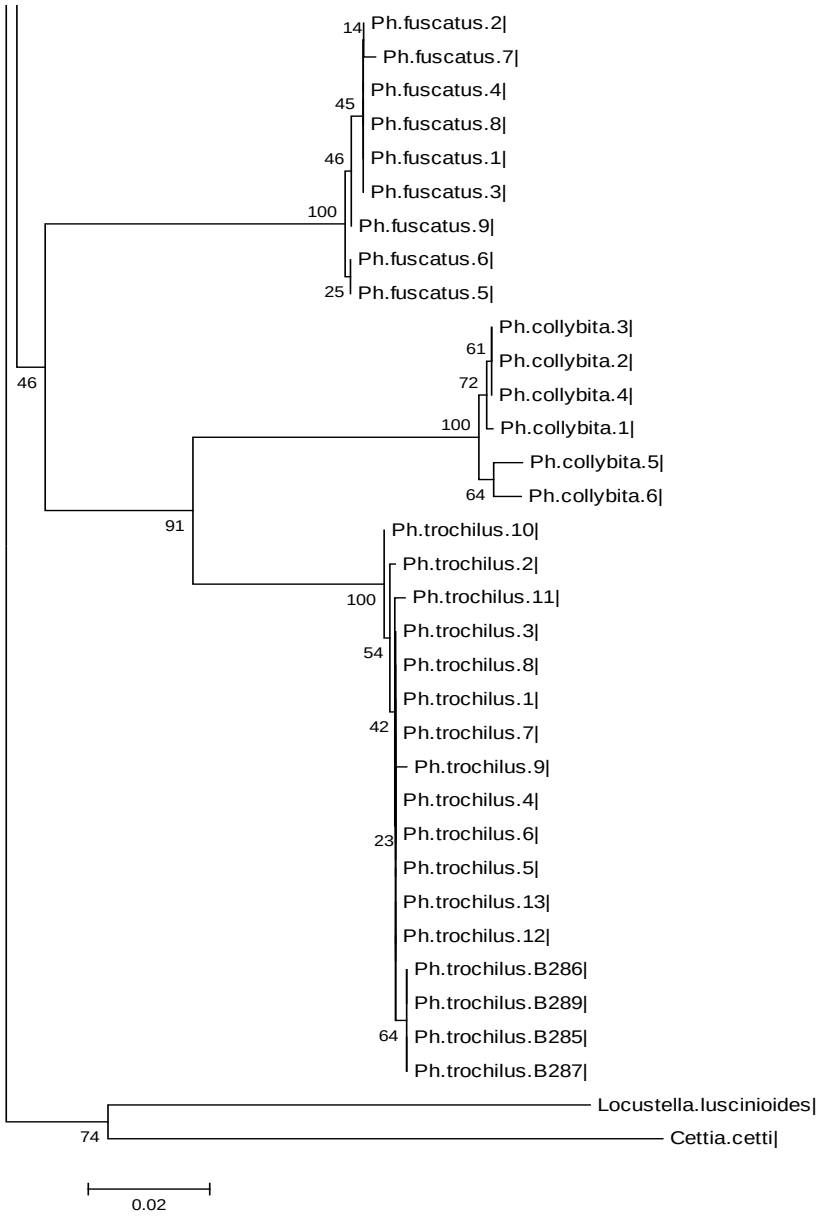

Figure 3.40. continued.

Six different haplotypes are observed for this species. Hap 2 is the most common haplotype observed in nine samples from three different countries (Russia, Sweden and Norway). Hap 3 is observed in all four samples from Turkey. Hap 1, 5, 6 are observed in Russia each in one sample and Hap 4 is observed in Norway (Fig. 3.41). These haplotypes show very shallow differentiation across their range.

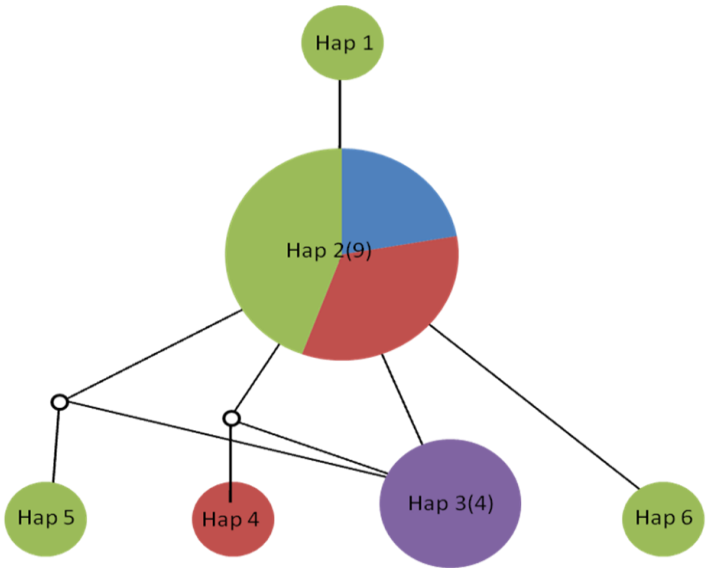

Figure 3.41. Haplotype network for *Phylloscopus trochilus*.

3.14. *Acrocephalus palustris* (Bechstein, 1798)

Eighteen different samples from four different countries (Russia, Turkey, Norway, and Sweden) are analyzed for this species (Fig. 3.42). Eight of analyzed samples are collected from Iğdır. Two of these eight samples were initially misidentified as *Acrocephalus scirpaceus* during ringing studies by their morphological characteristics (see below). This was due to the morphological similarities of *Acrocephalus palustris* and *Acrocephalus scirpaceus*, which can be expected, as they are sister species (Leisler et al., 1997).

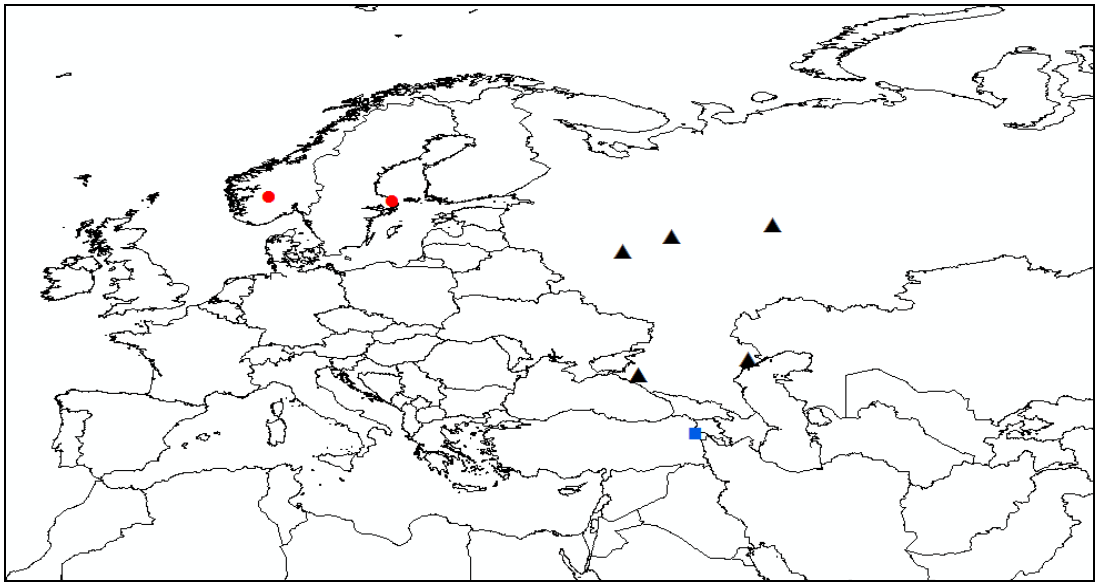

Figure 3.42. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

Comparing with the BOLD database, first closest match to Iğdır samples is *Acrocephalus palustris* with 100%, second with 99.81% and third with 99.63%. Phylogenetic tree contains 56 samples from nine different species and two outgroups *Bradypterus tacsanowskius* and *Cettia diphone* (Fig. 3.43). This tree shows that *A. palustris* is a monophyletic group sister to *A. scirpaceous.* All barcoded individuals from Iğdır (B24, B40, B43, B44, B45, B71 and B72) cluster closely in tree with other samples from BOLD. Among these, B72 and B71 are the individuals that were initially classified as *A. scirpaceous*, however in the phylogenetic tree they fall within the *A. palustris* clade.

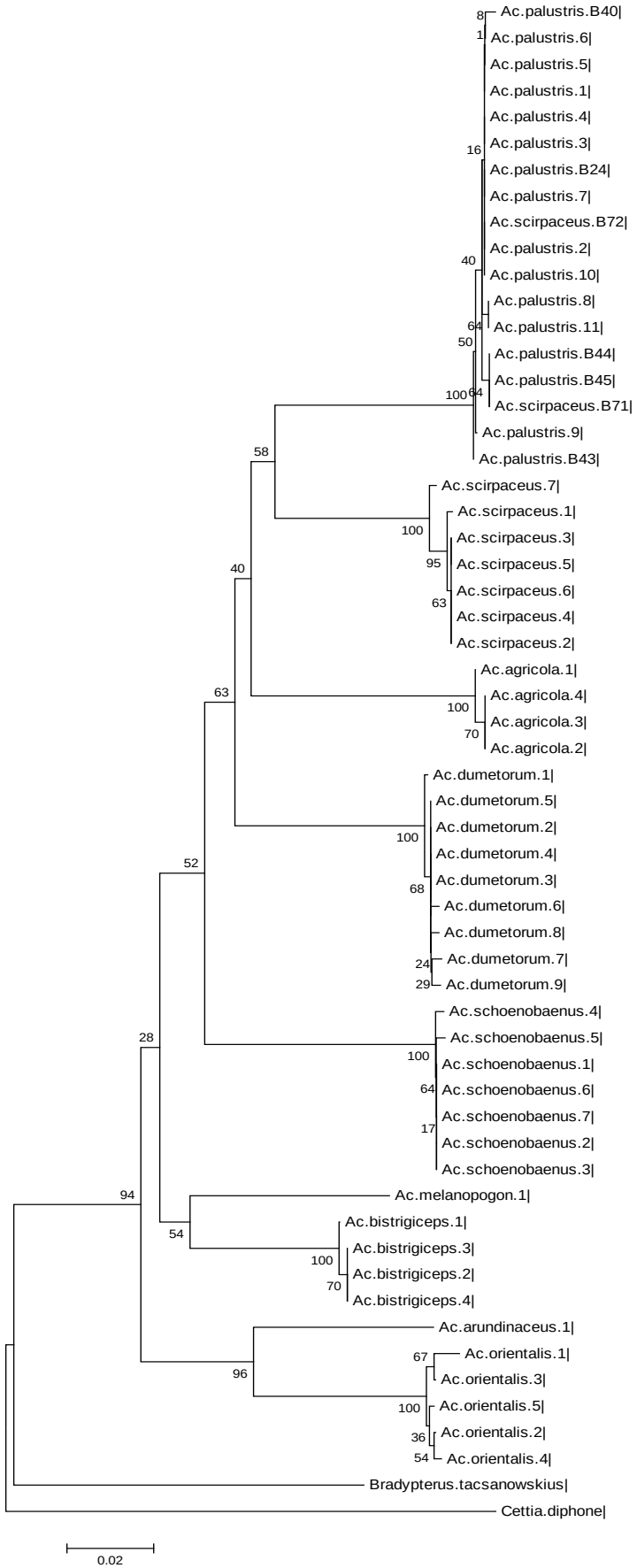

Figure 3.43. Neighbor-joining tree for *Acrocephalus palustris*.

Six different haplotypes are observed in this species, which form a star-like haplotype network for *Acrocephalus palustris* (Fig. 3.44). Hap 2 is the most common one that is seen in Turkey, Russia, Norway and Sweden for ten different samples. Remaining five haplotypes are only observed in one country, either Russia or Turkey. All five haplotypes differ by one base pair from Hap 2.

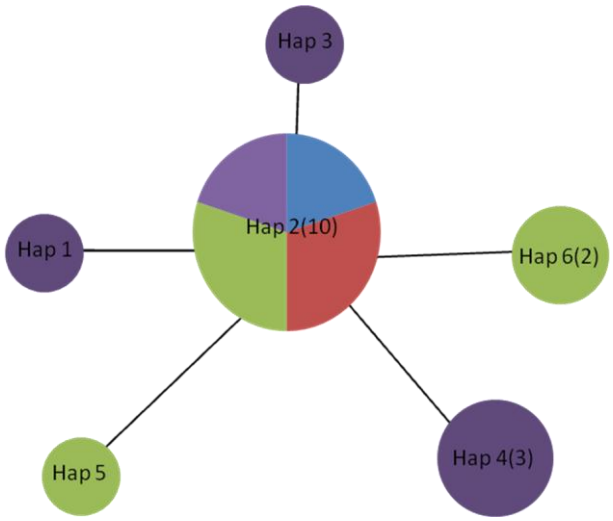

Figure 3.44. Haplotype network for *Acrocephalus palustris*.

3.15. *Locustella luscinioides* (Savi, 1824)

Four different samples from two countries (Russia and Turkey) are analyzed for this species (Fig. 3.45). There are two samples collected from Iğdır.

Figure 3.45. The locations for which COI Barcode Data were available from BOLD. The red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first closest match with Iğdır samples is *Locustella luscinioides* with 99.84% similarity. Second and third closest matches are with *Locustella fluviatilis* and 93.52% and 93.3% similarity, respectively. Phylogenetic tree contains 46 samples from nine species (Fig. 3.46). Two outgroups, *Sylvia atricapilla* and *Cettia cetti* are used in the neighbor-joining tree. Iğdır samples (B192, B194) are closely clustered with the other *Locustella luscinioides* sequence obtained from BOLD. *Locustella luscinioides* is seen as a sister clade with *Locustella fluviatilis*.

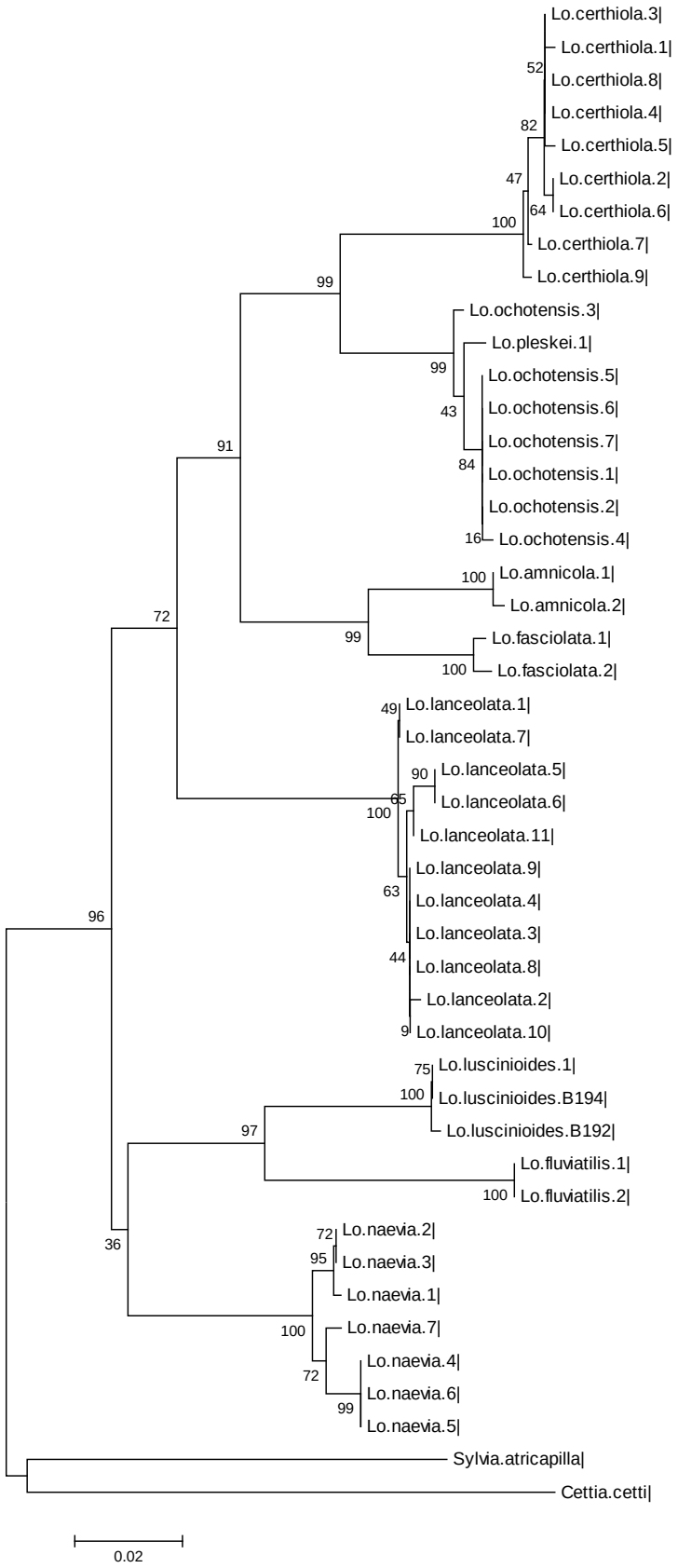

Figure 3.46. Neighbor-joining tree for *Locustella luscinioides*.

Two different haplotypes are observed for this species. Hap 1 is observed in Sweden and Turkey in two samples, whereas Hap 2 is observed only in Turkey (Fig. 3.47).

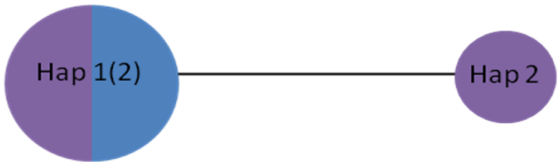

Figure 3.47. Haplotype network for *Locustella luscinioides*.

3.16. Genus *Sylvia*

Sylvia atricapilla, *Sylvia curruca* and *Sylvia nisoria* are studied from this genus. Phylogenetic tree contains 58 samples from eight species (Fig. 3.48). *Locustella luscinioides* and *Phylloscopus trochilus are* used in the neighbor-joining tree as outgroups.

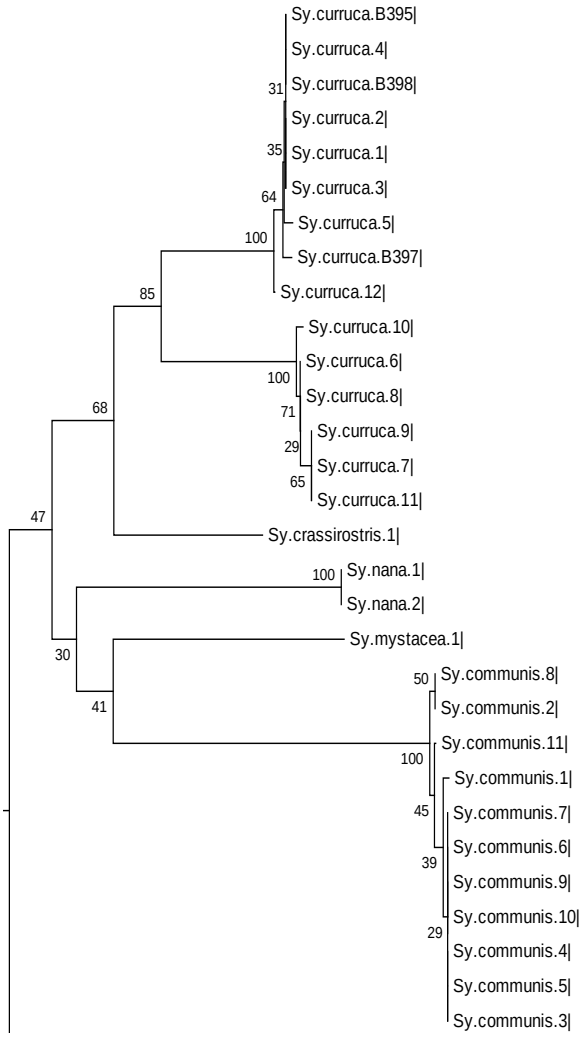

Figure 3.48. Neighbor-joining tree for *Sylvia*.

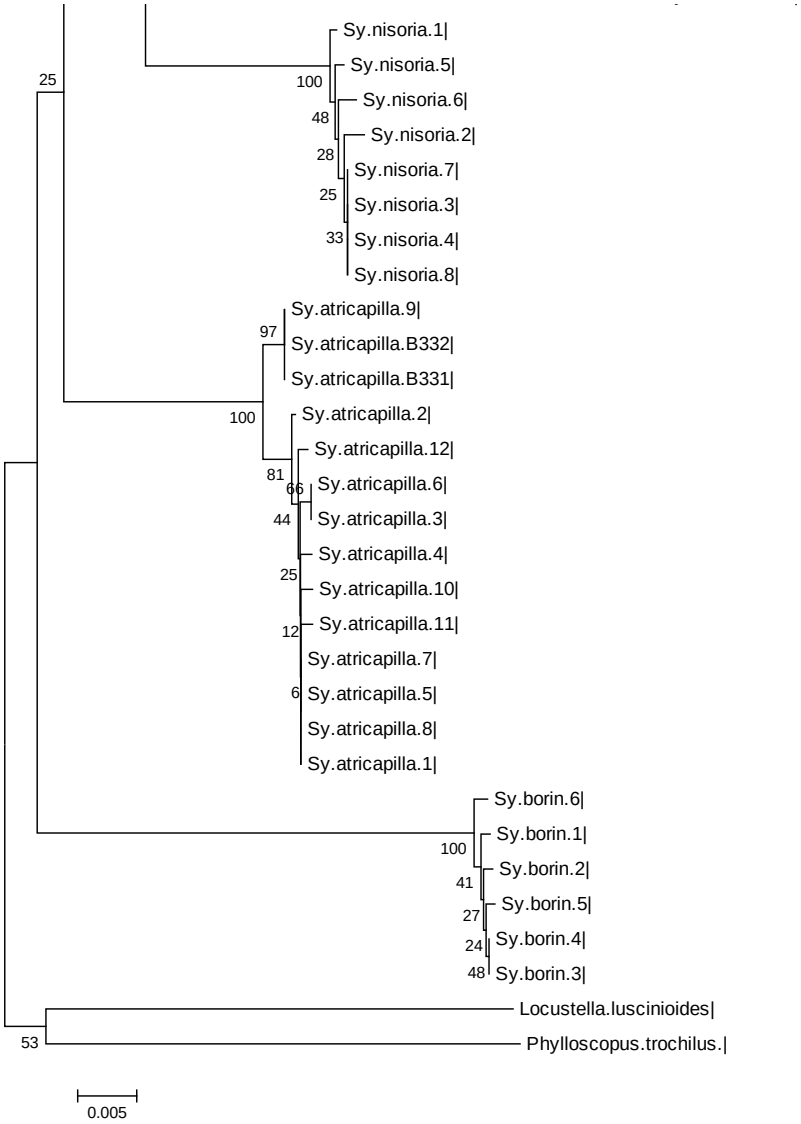

Figure 3.48. continued.

3.16.1. *Sylvia atricapilla* (Linnaeus, 1758)

Fourteen different samples from Russia, Turkey, Sweden, and Norway are analyzed for *Sylvia atricapilla* (Fig. 3.49). There are two samples collected from Iğdır.

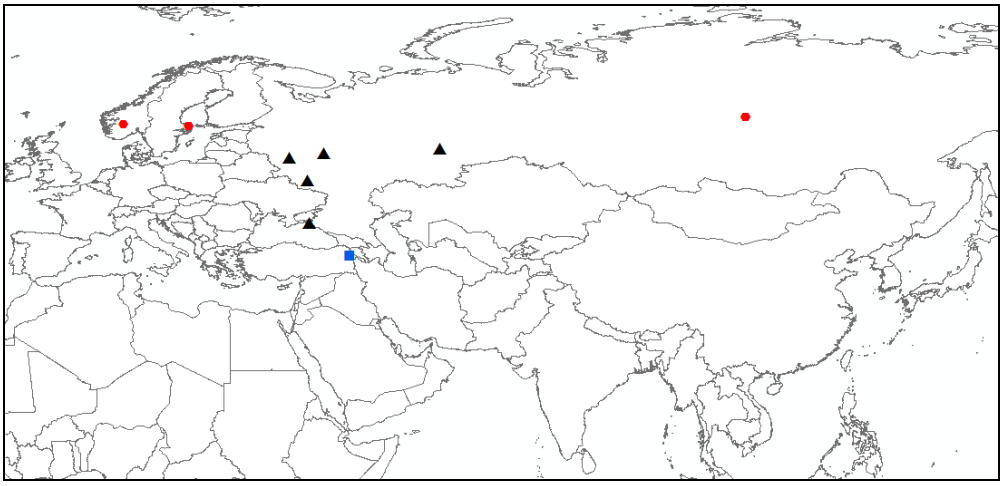

Figure 3.49. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first second and third closest matcher are with 100%, 99.81% and 99.03% similarity, respectively, to *Sylvia atricapilla*. *Sylvia borin* is used as an outgroup in the neighbor joining tree (Fig. 3.50). *Sylvia atricapilla* forms two clades in the neighbor-joining tree and Iğdır samples (B331, B332) are clustered in the same clade.

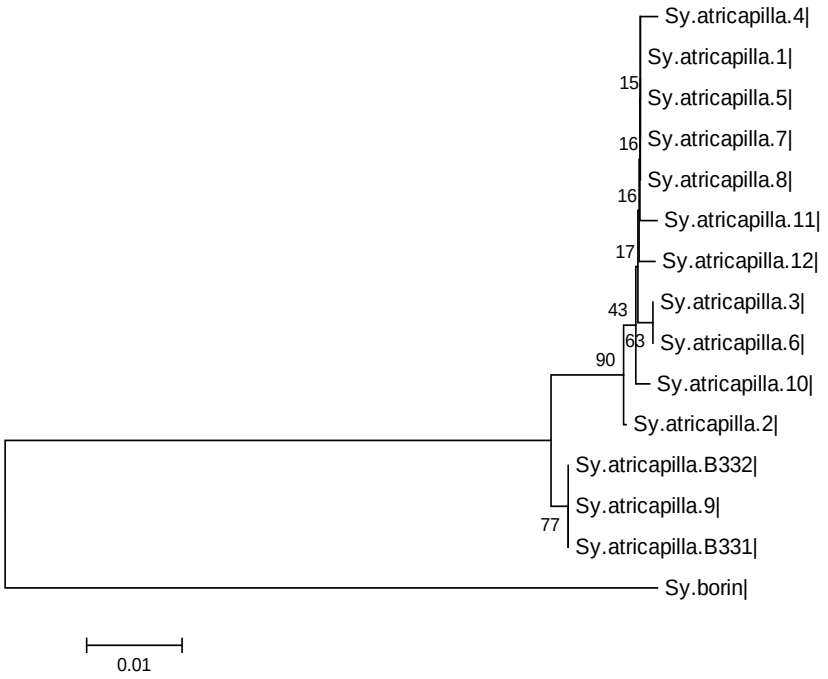

Figure 3.50. Neighbor-joining tree for *Sylvia atricapilla*

There are eight different haplotypes in this species. Hap 4 is the most common haplotype seen in four samples in Russia and Norway. Hap 1 is found in two samples from Turkey and one from Russia. Hap 5, 6, 7 and 8 are only observed in Russia (Fig. 3.51). The five base difference of Hap1 from the rest of the samples is also the reason why we observe two clades in the neighbor-joining tree, for *S. atricapilla.*

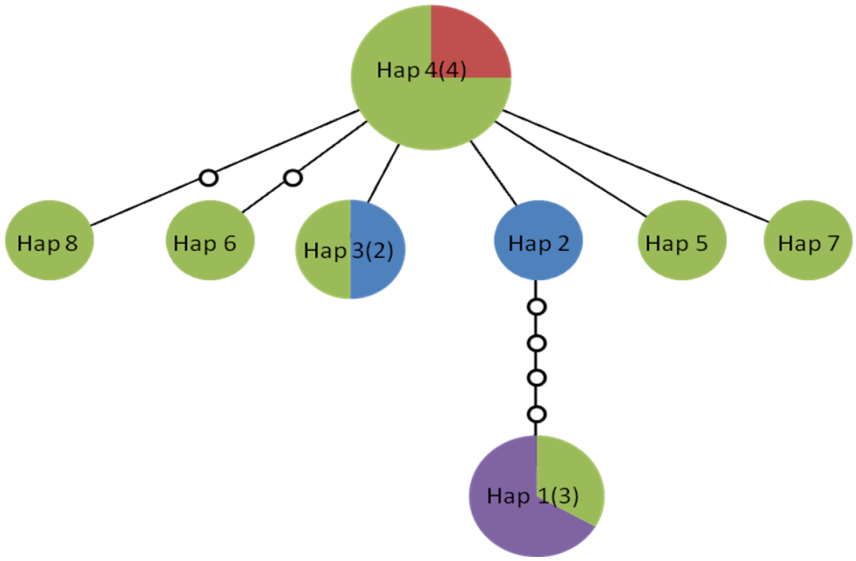

Figure 3.51. Haplotype network for *Sylvia atricapilla*.

3.16.2. *Sylvia nisoria* (Bechstein, 1792)

Nine different samples from Russia, Turkey, Kazakhstan, Sweden, and Norway are analyzed for *Sylvia nisoria* (Fig. 3.52). There is one sample collected from Iğdır.

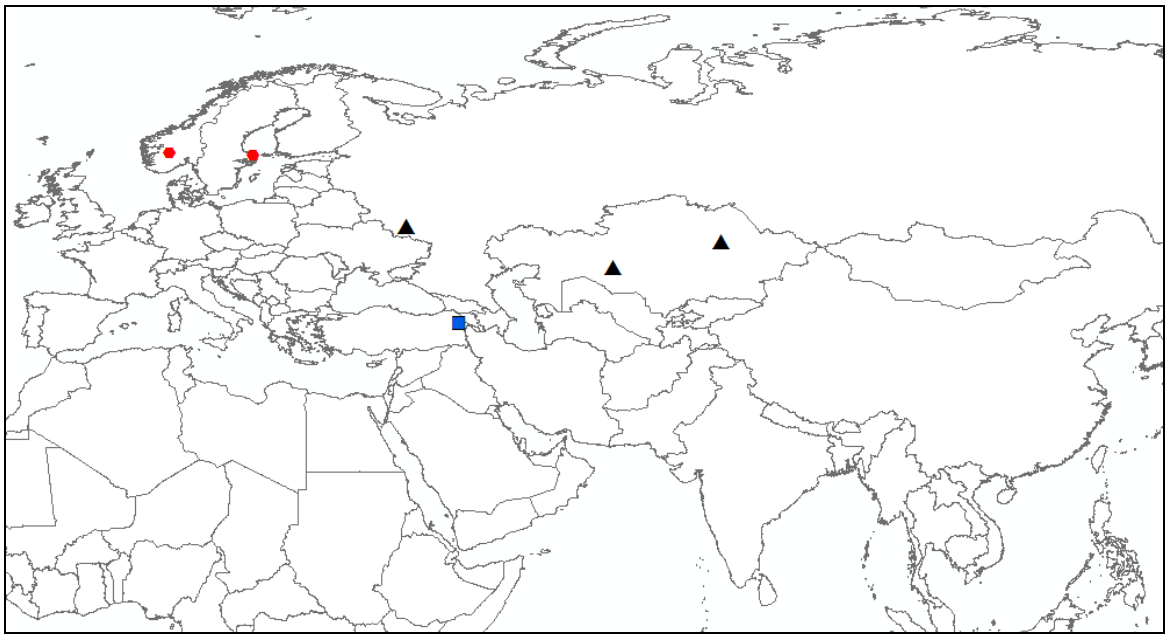

Figure 3.52. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first, second and third closest matches with Iğdır samples is *Sylvia nisoria* with 100% , 99.82% and 99.65% similarity, respectively. *Sylvia atricapilla* is used as an outgroup in the neighbor-joining tree (Fig. 3.53). This species forms a monophyletic clade.

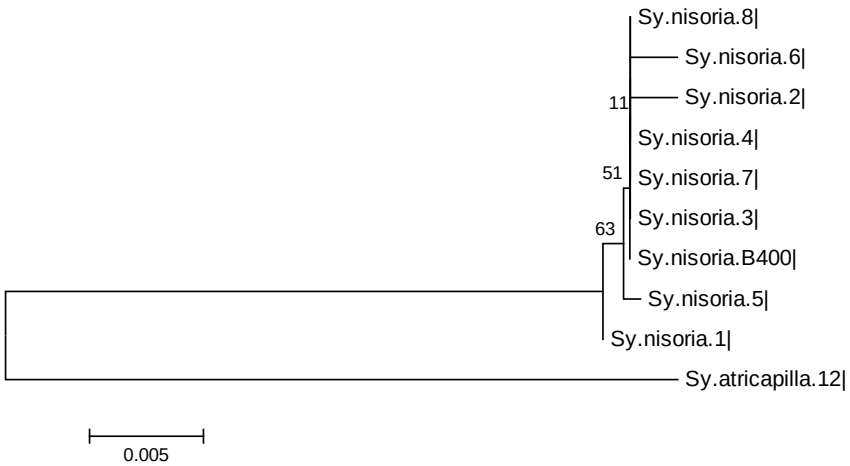

Figure 3.53. Neighbor-joining tree for *Sylvia nisoria*.

There are five different haplotypes in *Sylvia nisoria* with very low genetic differentiation. Hap 1 is observed in Turkey, Norway and Kazakhstan, in five samples. Hap 2 and Hap 3 are only observed in Russia, whereas Hap 4 and Hap 5 are observed only in Sweden (Fig. 3.54).

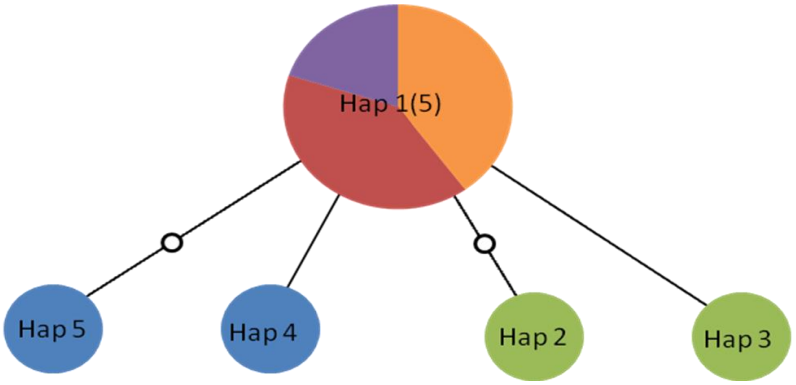

Figure 3.54. Haplotype network for *Sylvia nisoria*.

3.16.3. *Sylvia curruca* (Linnaeus, 1758)

Fifteen different samples from Russia, Turkey, Mongolia, Kazakhstan, Sweden, and Norway are analyzed for *Sylvia curruca* (Fig. 3.55). There are three samples collected from Iğdır.

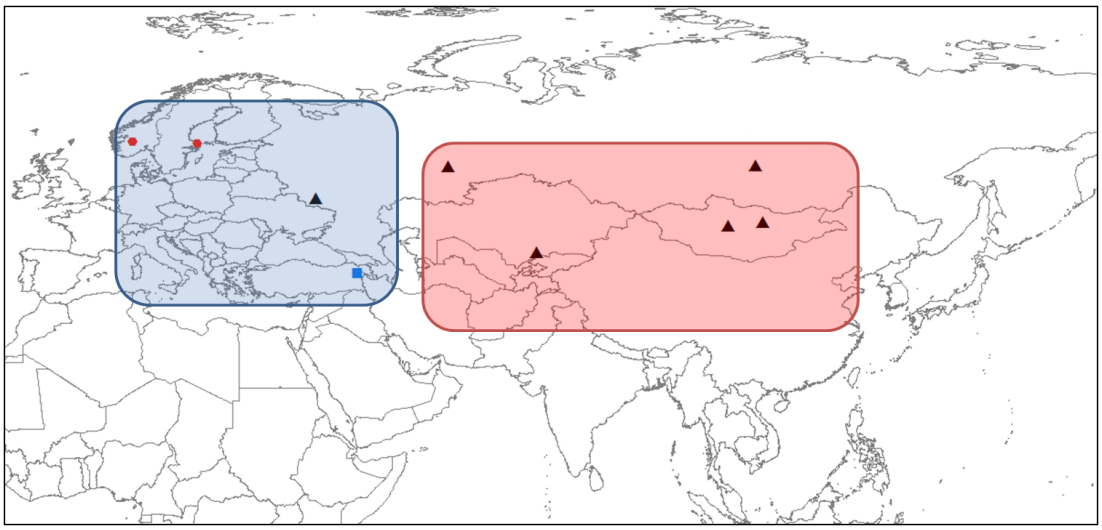

Figure 3.55. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır. Hap 1, 2, 3 are indicated by red and Hap 4, 5, 6, 7 are indicated with blue areas.

A comparison with the BOLD database shows first, second and third closest matches with Iğdır samples is *Sylvia curruca* with 100%, 99.84% and 99.65% similarity respectively. *Sylvia borin* is used as an outgroup in the neighbor-joining tree (Fig. 3.56). *Sylvia curruca* forms two clades in the neighbor-joining tree and Iğdır samples (B335, B337 and B338) are clustered in the same clade. Different clades indicate eastern and western parts of the species' range.

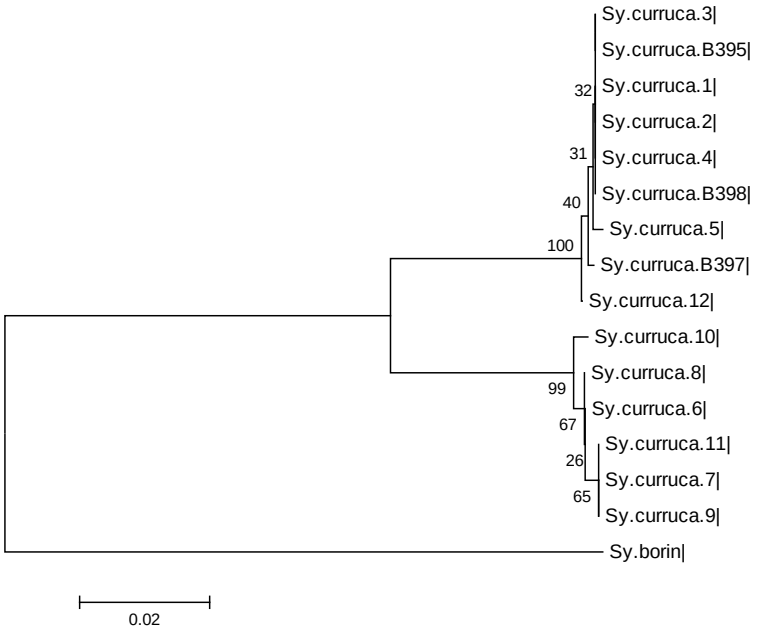

Figure 3.56. Neighbor-joining tree for *Sylvia curruca*.

This differentiation of the haplotypes into two clades in the neighbor-joining tree is also observed as the presence of two groups of haplotypes in the *Sylvia curruca* network, with a 32 base pair difference (Fig. 3.57). The first group contains nine samples from Russia, Turkey, Norway, and Sweden. This group contains four different haplotypes. Hap 4 is observed in all four countries. Hap 6 and 7 are observed in Russia, and Hap 5 is observed only in Turkey. The second group is composed of six samples from Russia, Kazakhstan, and Mongolia. Hap 2 is the most common haplotype in this group, which is observed in all three countries. Hap 1 is observed only in Russia whereas Hap 3 is observed only in Kazakhstan.

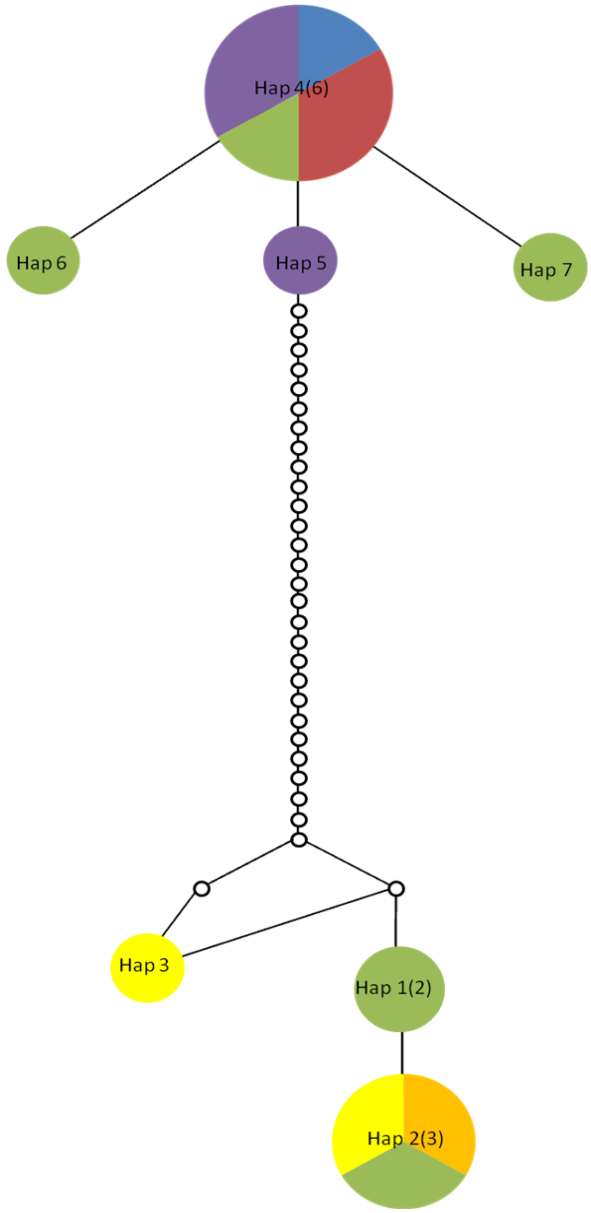

Figure 3.57. Haplotype network for *Sylvia curruca*.

3.17. *Muscicapa striata* (Pallas, 1764)

Twelve samples from five countries (Russia, Kazakhstan, Sweden, Norway and Turkey) are analyzed for this species (Fig. 3.58). There is one sample collected from Iğdır.

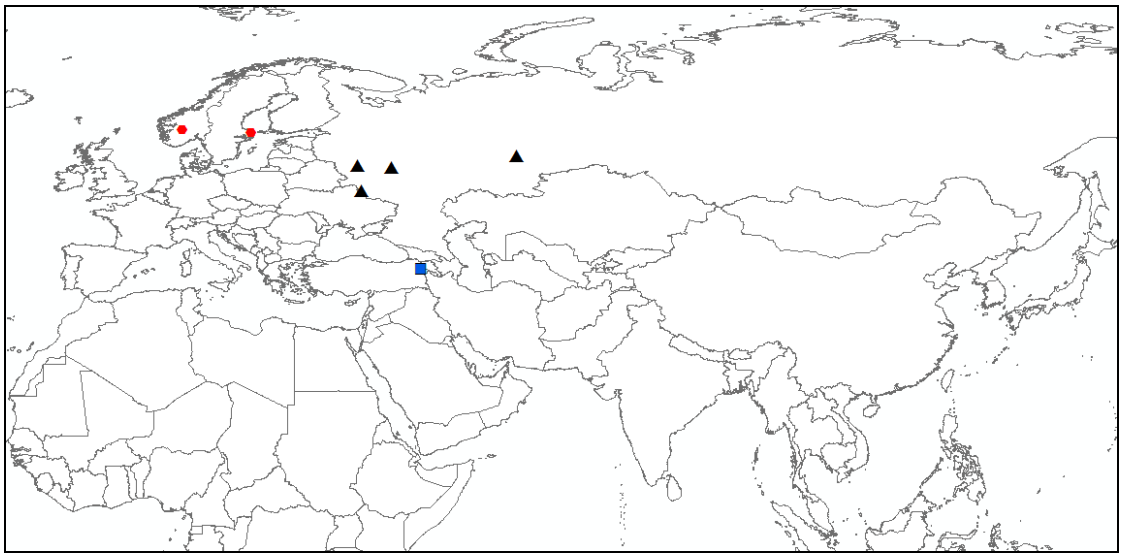

Figure 3.58. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first closest match with Iğdır samples is *Muscicapa striata* with 100%, second with 99.84% and third with 99.69% similarity. Phylogenetic tree contains 30 samples from four species (Fig. 3.59). Two outgroups, *Luscinia luscinia* and *Ficedula parva* are used in the neighbor-joining tree. *Muscicapa striata* formed a monophyletic clade in tree and Iğdır sample (B250) is clustered in the clade.

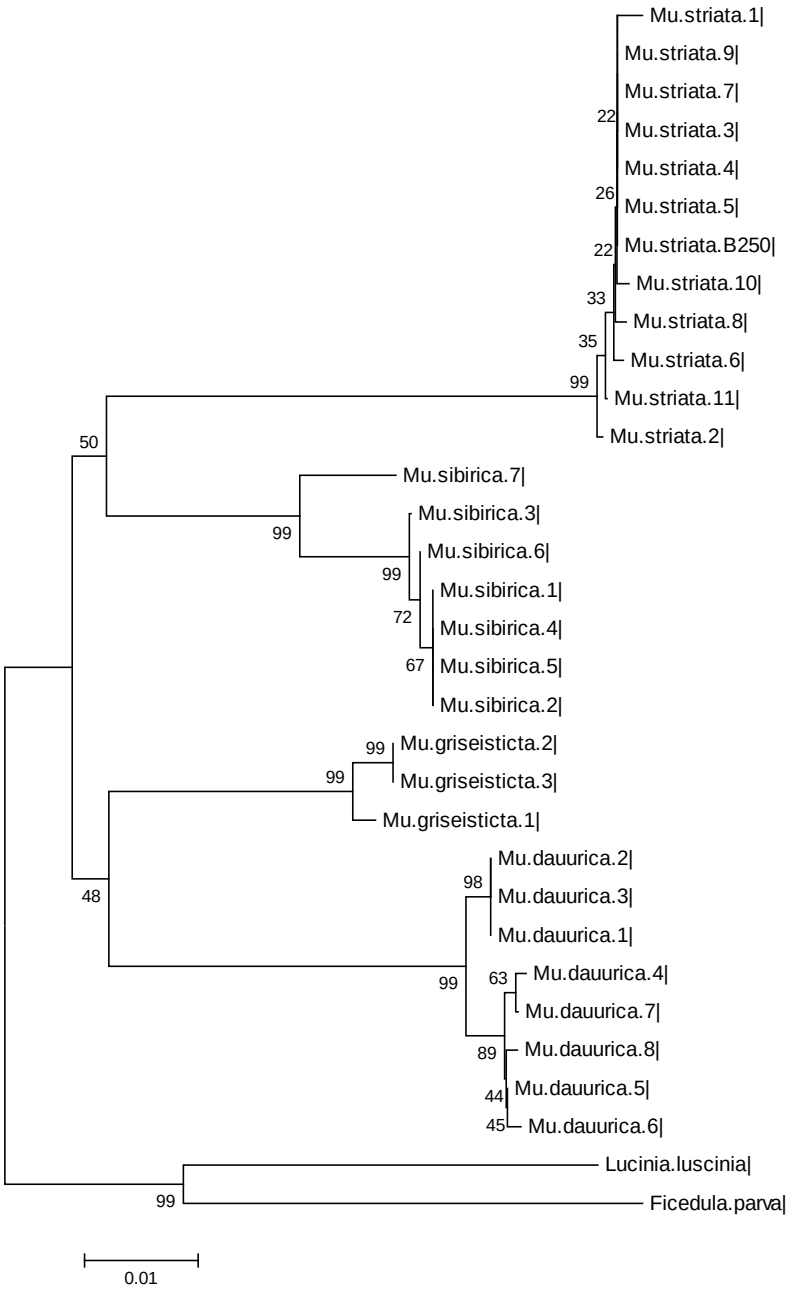

Figure 3.59. Neighbor-joining tree for *Muscicapa striata*.

Among all seven haplotypes, Hap 1 is the most commonly seen haplotype that is observed in three different countries (Russia, Sweden and Turkey) with six different samples (Fig. 3.60). Remaining six haplotypes are all observed only in one country, three in Russia, two in Norway and one in Kazakhstan. Although seven haplotypes are observed, there is a maximum of four base pairs of difference in the most remote parts of the network.

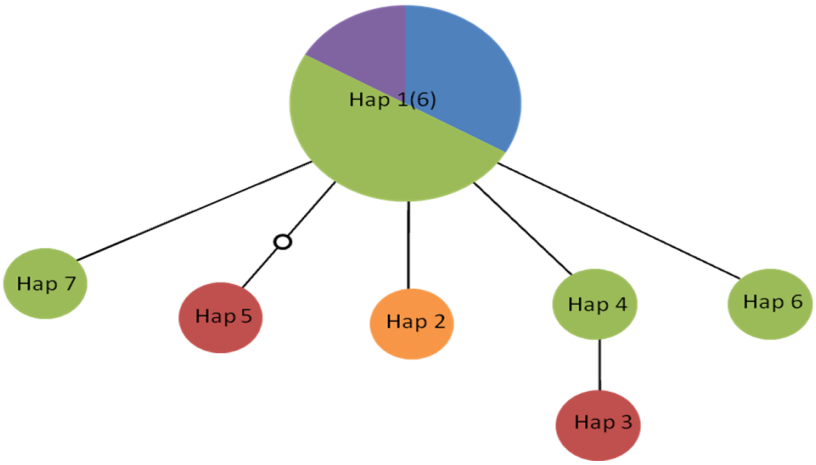

Figure 3.60. Haplotype network for *Muscicapa striata*.

3.18. *Ficedula parva* (Bechstein, 1792)

Eight different samples from five different countries (Russia, Turkey, Sweden, South Korea and Norway) are analyzed for this species. There are two samples collected from Iğdır. Two major groups of haplotypes are observed on the map (Fig. 3.61). One group is found in Europe, Anatolia and Middle Asia and other is observed in South Korea.

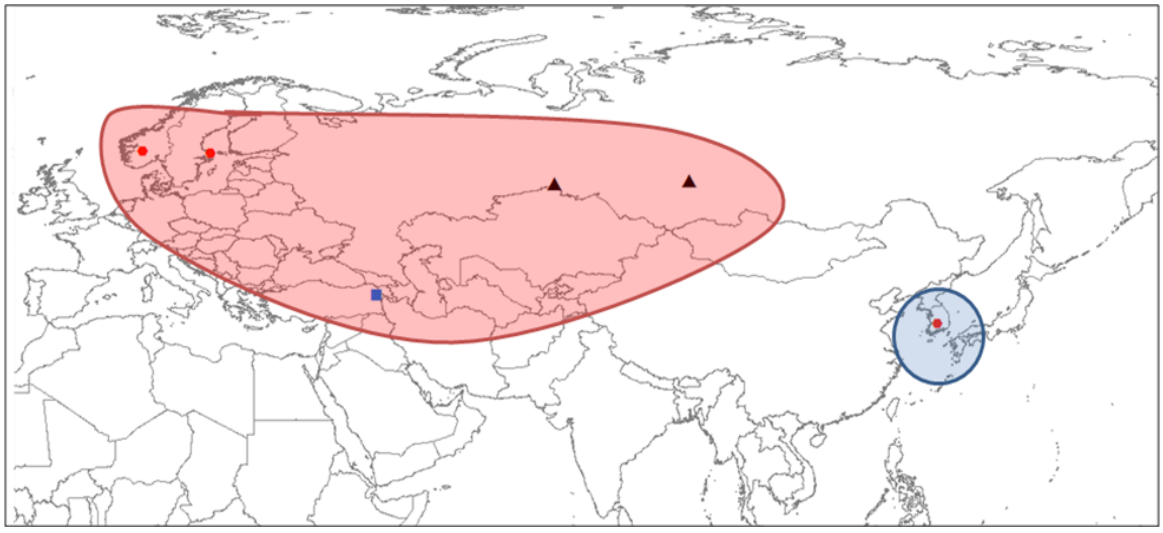

Figure 3.61. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır. Blue and red areas indicate Hap 3 and Hap 1, 2 respectively.

A comparison with the BOLD database shows first closest match is *Ficedula parva* with 100% similarity. Second closest match is *Ficedula albicilla* with 94.52 % similarity. Third closest match is *Ficedula parva* with 94.34% similarity. Phylogenetic tree contains 52 samples and 10 species (Fig. 3.62). Two outgroups *Luscinia megarhynchos* and *Erithacus rubecula* are used in the neighbor-joining tree. Iğdır samples (B144 and B145) are clustered in the *Ficedula parva* clade. *Ficedula parva* is seen as sister to the *Ficedula albicilla* clade. *Ficedula parva* sample from South Korea (Fi. parva 6) is clustered in the *Ficedula albicilla* clade.

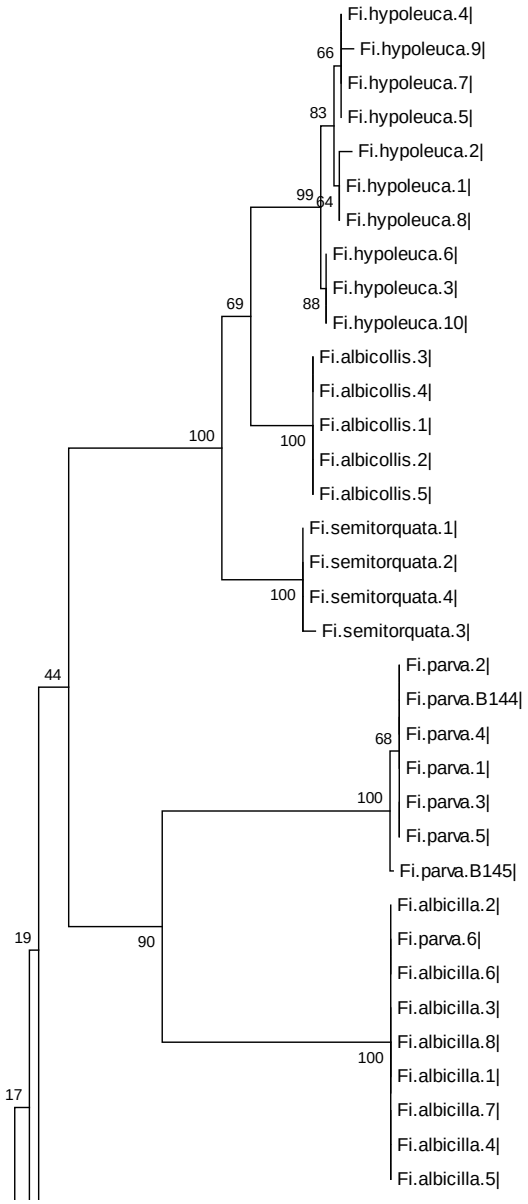

Figure 3.62. Neighbor-joining tree for *Ficedula parva*.

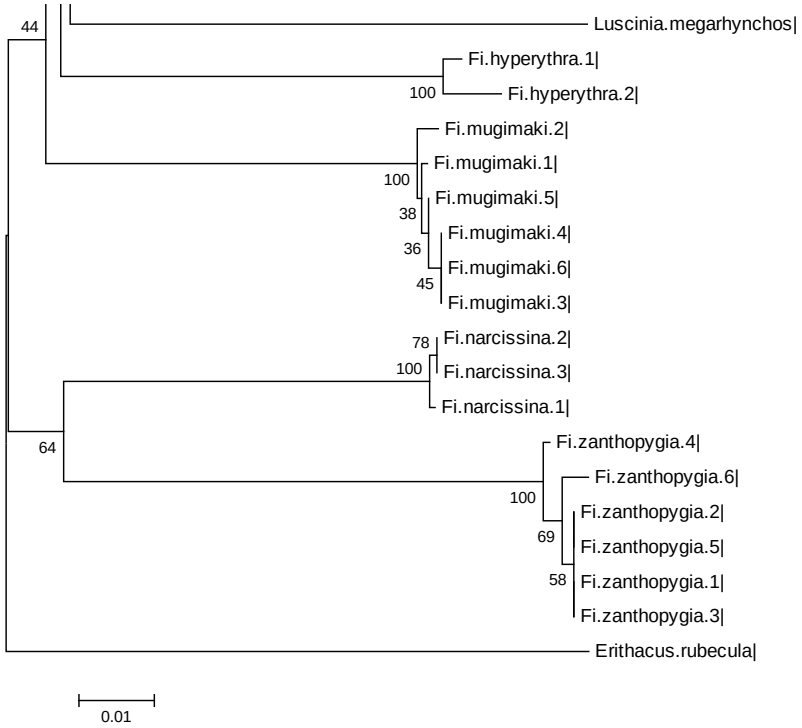

Figure 3.62. continued.

Three haplotypes are observed for this species. Hap 1 is the most commonly observed haplotype seen in four different (Russia, Sweden, Turkey and Norway) countries (Fig. 3.63). Hap 2 is a new haplotype seen only in Iğdır. Hap 3, which is very divergent from other haplotypes by 36 base pairs, is only observed in South Korea in one sample.

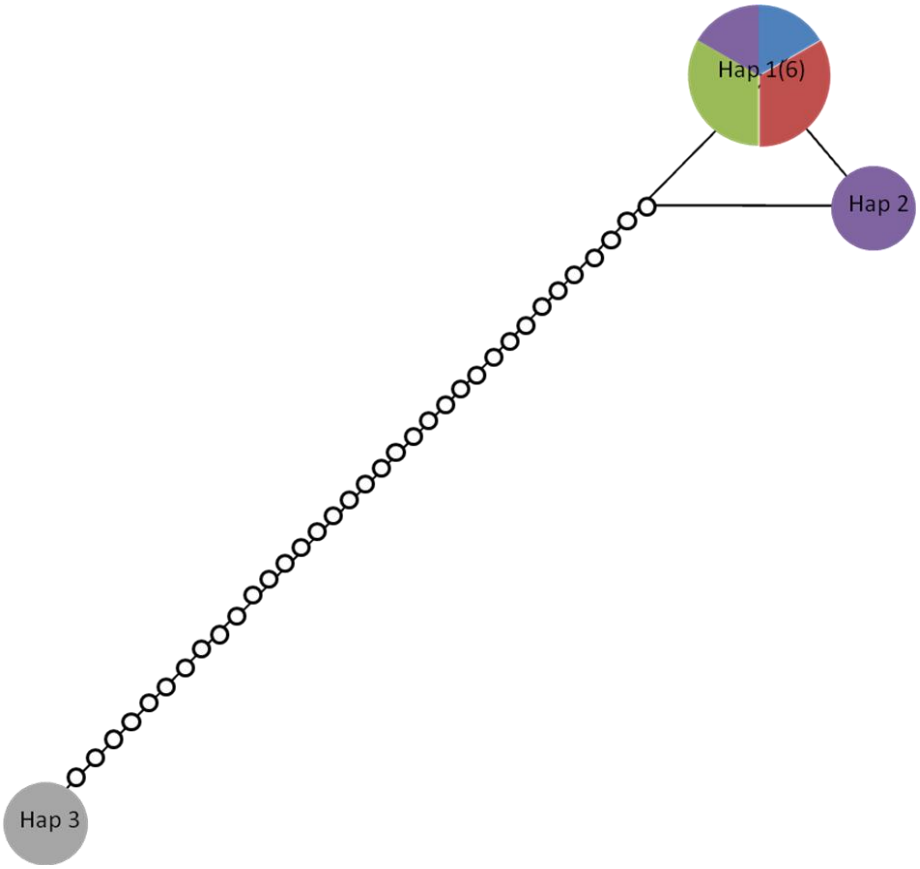

Figure 3.63. Haplotype network for *Ficedula parva*.

3.19. *Erithacus rubecula* (Linnaeus, 1758)

Thirteen different samples from four different countries (Russia, Turkey, Sweden and Norway) are analyzed for this species (Fig. 3.64). There are two samples collected from Iğdır.

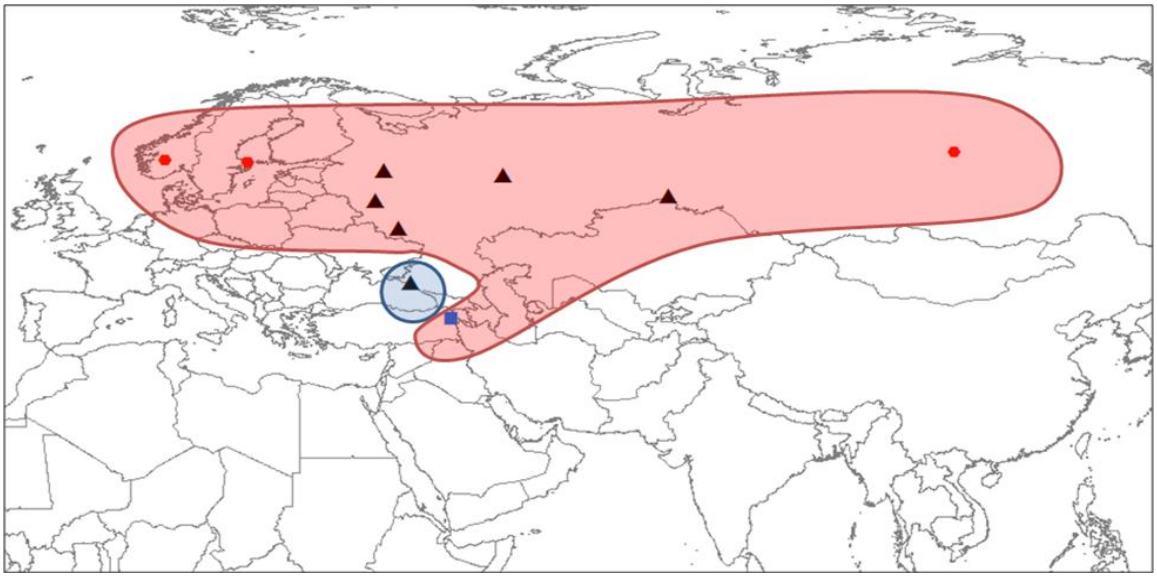

Figure 3.64. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır. Blue area indicates Hap 6 and other haplotypes' geographical distributions are indicated with red.

A comparison with the BOLD database shows closest matches first with 100%, second with 99.83% and third with 99.66% similarity to *Erithacus rubecula*. Phylogenetic tree contains 21 samples and two species (Fig. 3.65). Two outgroups, *Ficedula parva* and *Luscinia sibilans* are used in the neighbor-joining tree. Two main clades are observed in neighbor joining tree which is also confirmed by different colored areas in the map (Fig. 3.64). All Iğdır samples (B139, B141) are clustered in the first clade. Second clade contains only one sample which is from Russia.

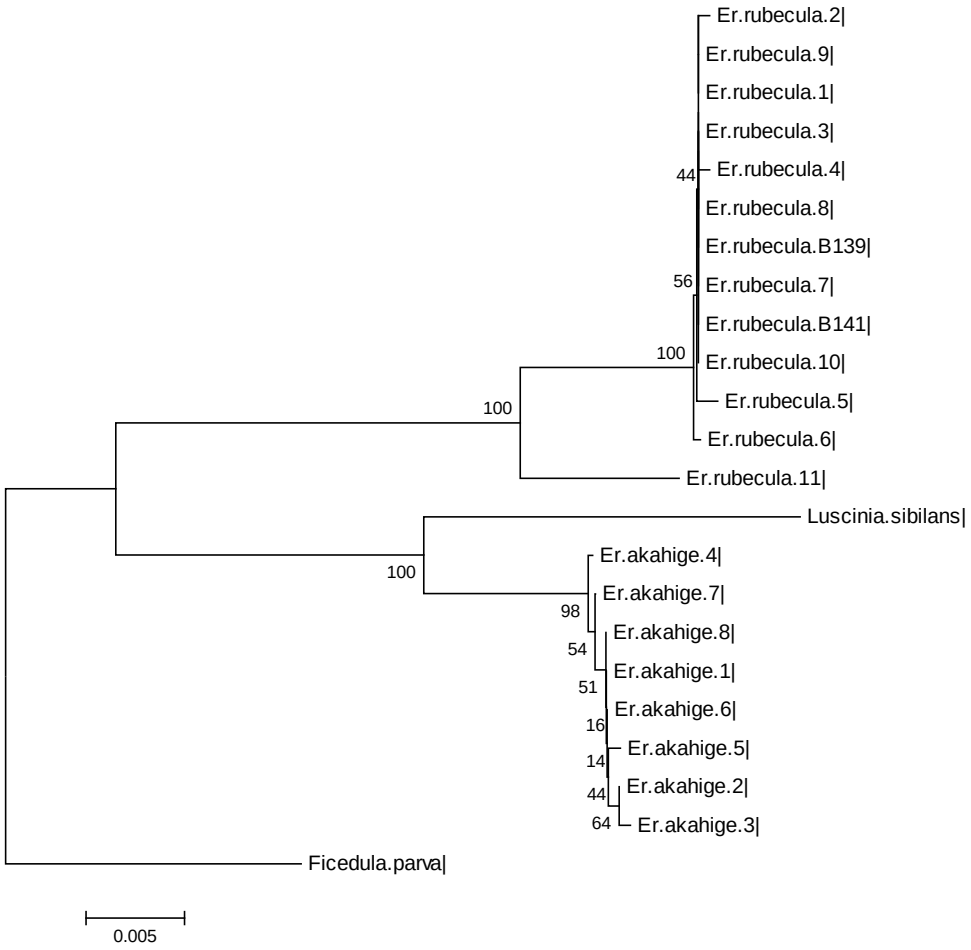

Figure 3.65. Neighbor-joining tree for *Erithacus rubecula*.

Six different haplotypes are observed for *Erithacus rubecula*. According to haplotype network, Hap 1 is most commonly observed haplotype, which seen in eight different samples from four different (Russia, Sweden, Turkey and Norway) countries (Fig. 3.66). Three, one and one haplotypes are only observed in Russia, Norway and Sweden, respectively. Hap 6, which is observed in Russia, is highly divergent from the other samples, differentiated by 28 base pairs. That difference is why this sample (Er. rubecula.11) clusters separately from the rest of the *E. rubecula* samples in the neighbor-joining tree, above. The geographic location of this sample is indicated as a blue, and the distribution of the remaining haplotypes are indicated as a red area in Fig. 3.64.

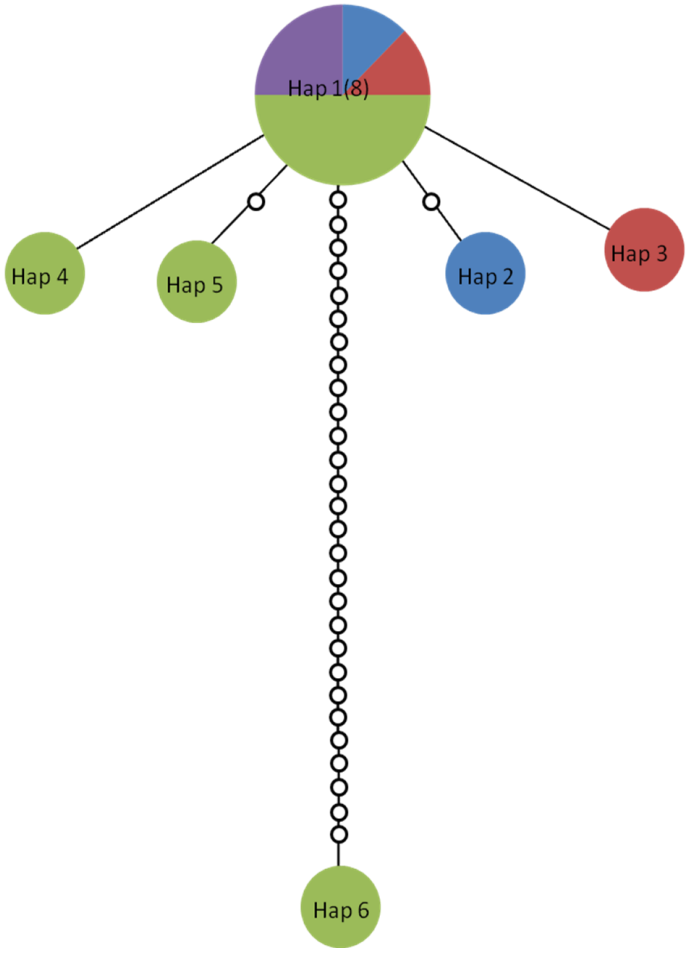

Figure 3.66. Haplotype network for *Erithacus rubecula*.

3.20. *Luscinia svecica* (Linnaeus, 1758)

Fourteen samples from four different countries (Russia, Turkey, Norway, and Sweden) are analyzed for *Luscinia svecica*. There is one sample which is collected from Iğdır (Fig. 3.67).

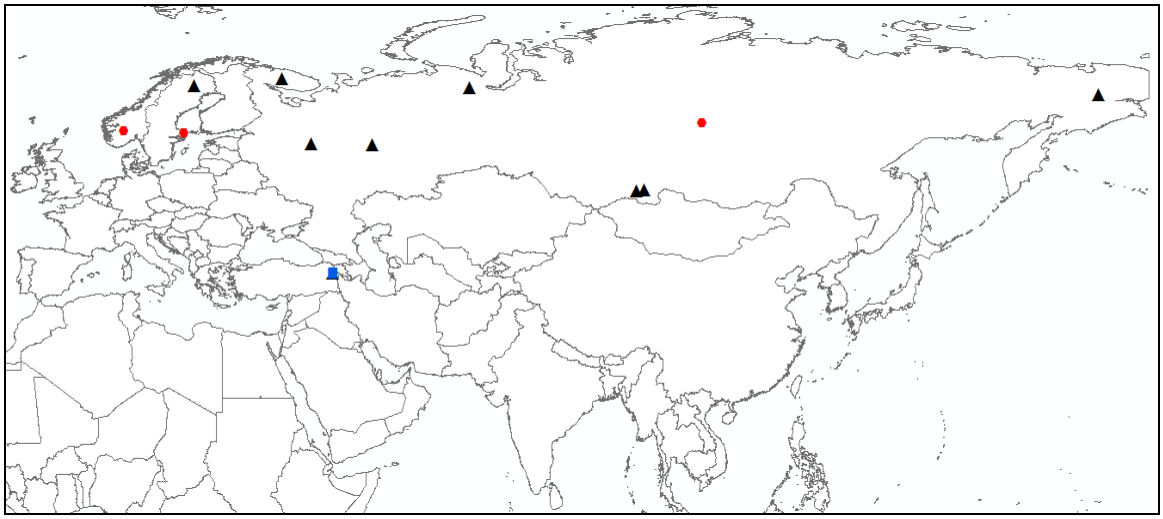

Figure 3.67. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first, second and third closest matches are with *Luscinia svecica* with 100%, 99.8% and 99.59% similarity, respectively. Phylogenetic tree contains 52 samples from six species (Fig. 3.68). Two outgroups *Erithacus rubecula* and *Ficedula parva* are used in the neighbor-joining tree. In the tree, Iğdır sample (B209) clustered closely with the rest of the barcodes from BOLD, from the entire northern Palearctic.

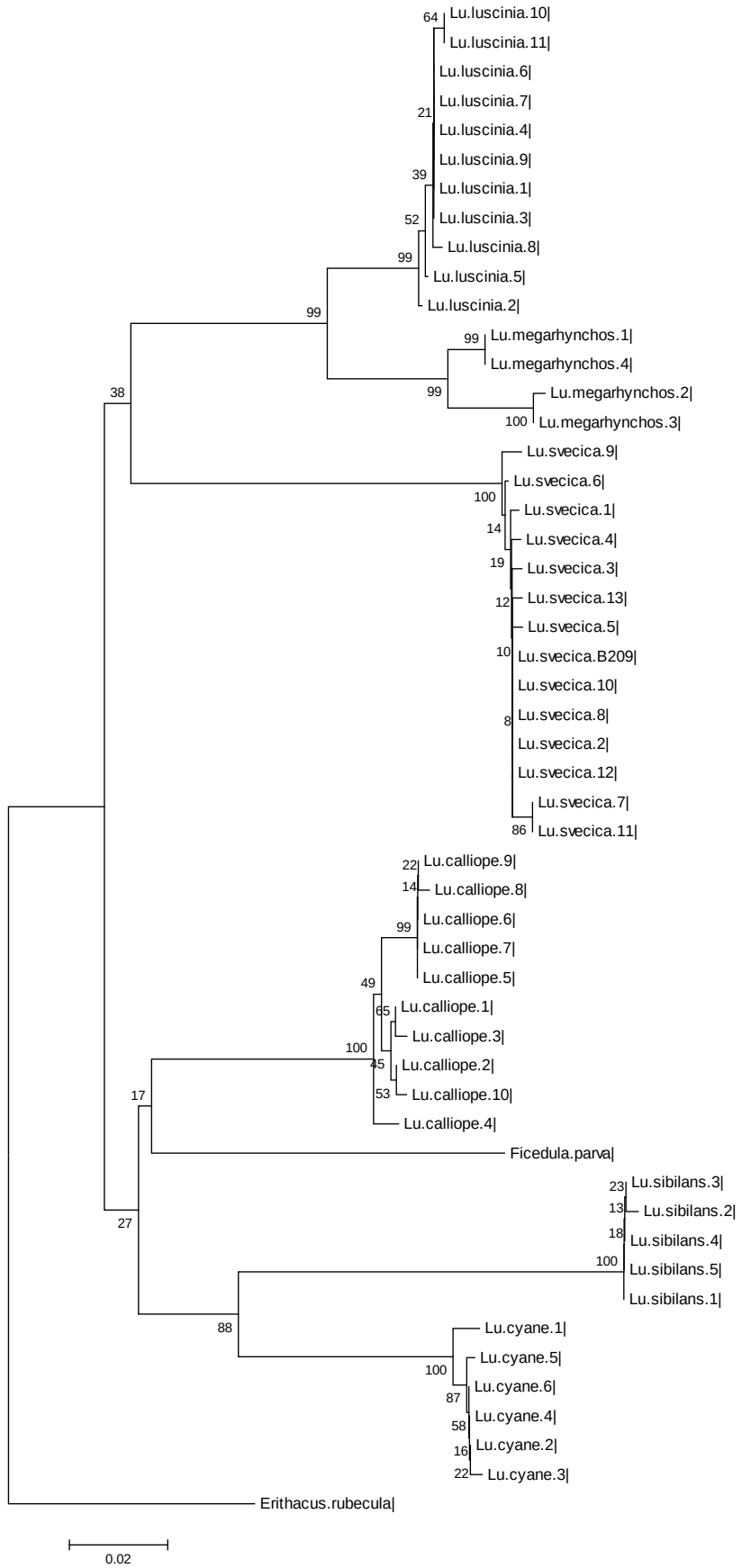

Figure 3.68. Neighbor-joining tree for *Luscinia svecica*.

Nine different haplotypes are observed for this species. Hap 2 is the most common haplotype observed in three countries (Russia, Sweden and Turkey) in five samples (Fig. 3.69). Haplotypes 1, 3, 4, 6 and 8 are only observed in Russia. Hap 5 and Hap 7 is observed in Sweden and Hap 9 is observed only in Norway. Although nine haplotypes are observed, there is a maximum of five base pairs of difference between the most remote parts of the network.

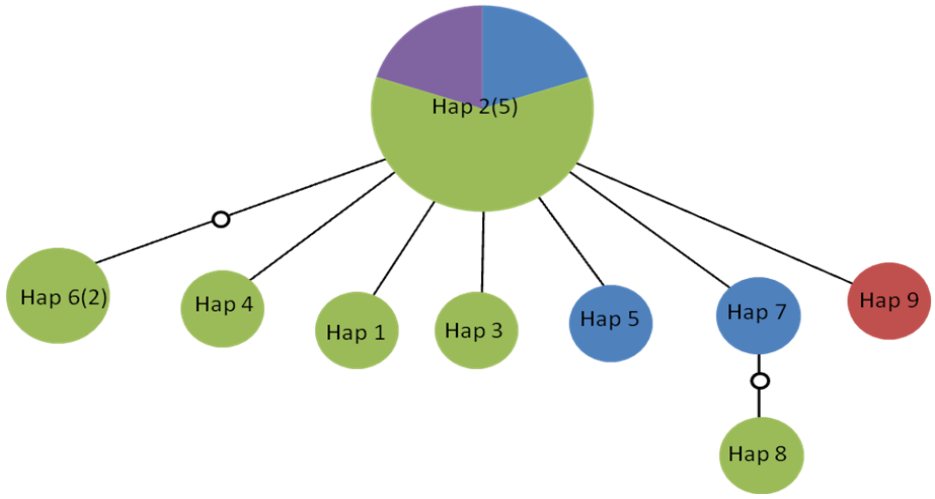

Figure 3.69. Haplotype network for *Luscinia svecica*.

3.21. *Phoenicurus phoenicurus* (Linnaeus, 1758)

Eighteen different samples from six different countries (Russia, Turkey, Kazakhstan, Mongolia, Norway and Sweden) are analyzed for this species (Fig. 3.70). There are five samples collected from Iğdır.

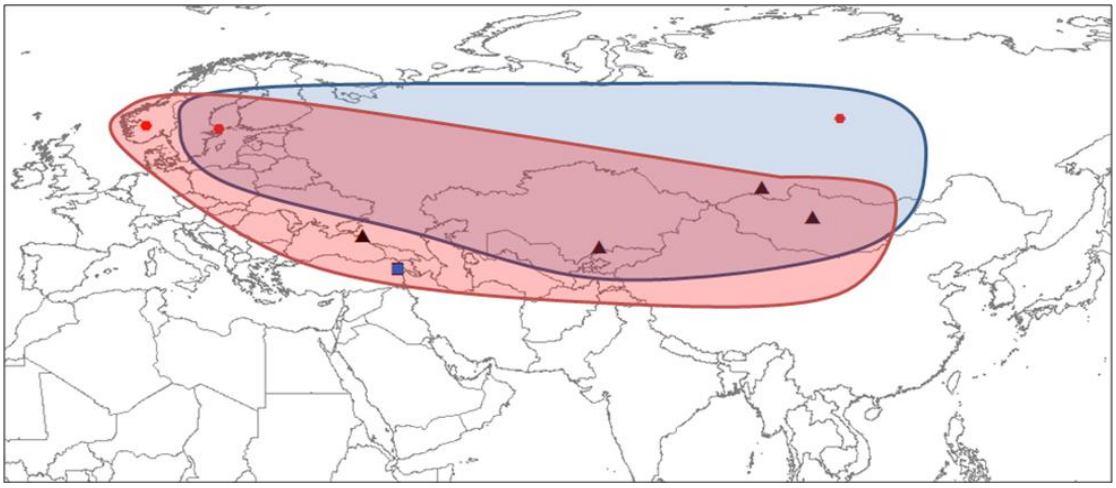

Figure 3.70. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır. Hap 5, 6, 7, 8, 9, 10 are indicated with red and Hap 1, 2, 3, 4 are indicated with blue.

A comparison with the BOLD database shows first second and third closest matcher are with 99.85% 99.7% and 99.69% similarity, respectively, to *Phoenicurus phoenicurus*. Phylogenetic tree contains 45 samples from five species (Fig. 3.71). Two outgroups *Luscinia megarhynchos* and *Erithacus rubecula* are used in the neighbor-joining tree. *Phoenicurus phoenicurus* samples form two clades, where the samples that we barcoded from Turkey (B295, B296, B297, B298, B299) fall within one of these clades.

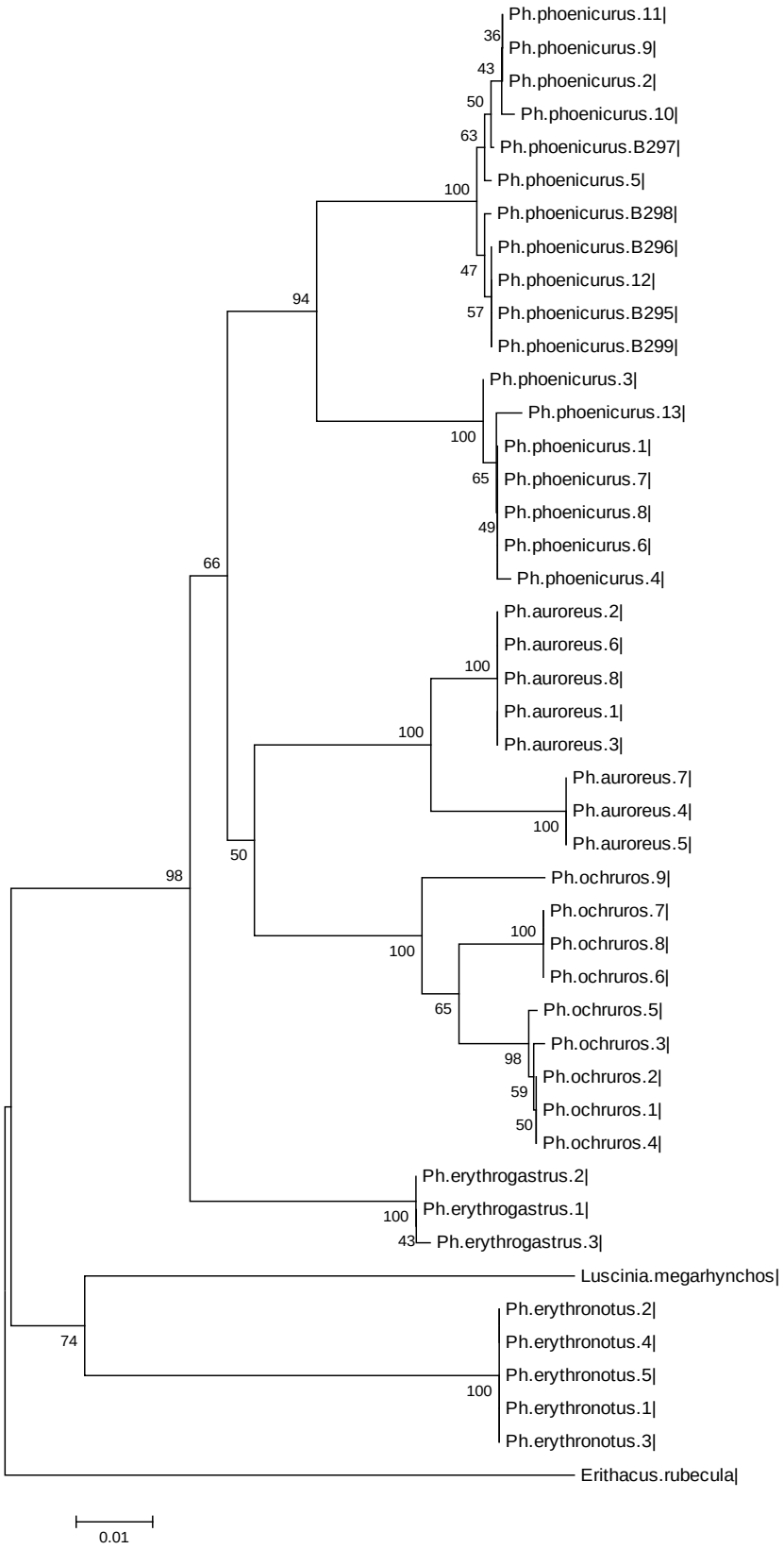

Figure 3.71. Neighbor-joining tree for *Phoenicurus phoenicurus*.

Ten different haplotypes are observed for this species. There are two groups of haplotypes with 27 base pair differences in the *Phoenicurus phoenicurus* network (Fig. 3.72). First group is composed of 11 samples from Russia, Turkey, Norway, Mongolia and Kazakhstan. This group contains six different haplotypes. Hap 7, 9 and 10 are from Turkey. Hap 7 and 10 are new haplotypes observed in Turkey. Second group contains seven samples from Russia, Norway, and Sweden. Hap 2 is most common haplotype in this group, which is observed in all three countries. Hap 1, Hap 2, Hap 3 and Hap 4 are indicated with blue, and Hap 5, Hap 6, Hap 7, Hap 8, Hap 9 and Hap 10 are indicated with red to indicate the geographical distribution of these haplogroups on Figure 3.68.

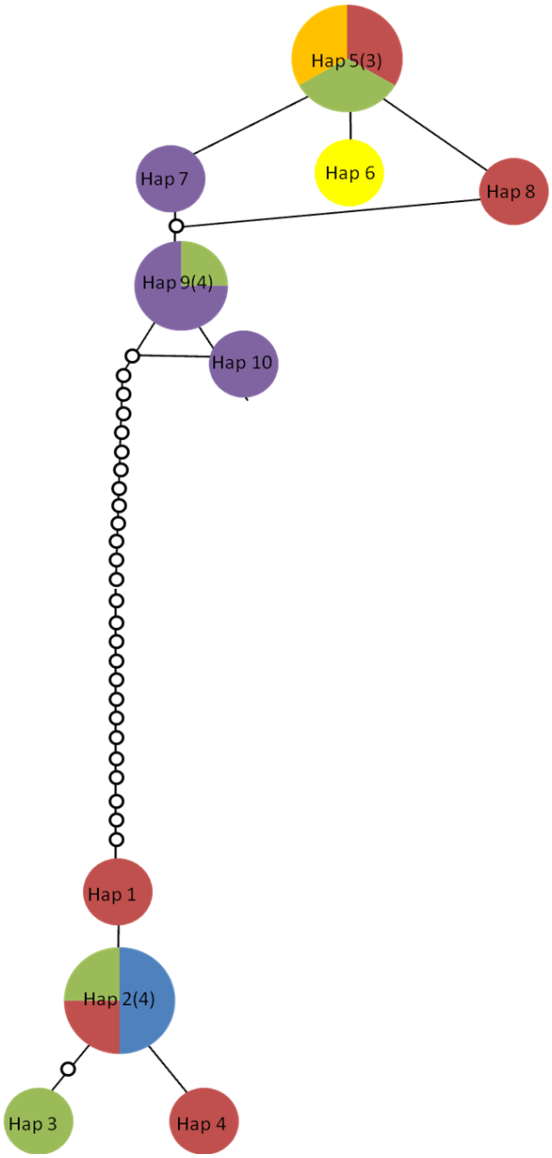

Figure 3.72. Haplotype network for *Phoenicurus phoenicurus*.

3.22. *Oenanthe hispanica* (Linnaeus, 1758)

Two samples, with one from Turkey are studied (Fig. 3.73). The other sample's location information was not available in the BOLD database. Both samples' COI regions are identical.

Figure 3.73. The locations for which COI Barcode Data were available from BOLD. The blue square indicates Iğdır.

A comparison with the BOLD database shows first closest match with Iğdır samples is *Oenanthe hispanica* with 100% similarity. Second and third closest matches are with *Oenanthe pleschanka* and 99.82% and 99.64% similarity, respectively. The phylogenetic tree contains 64 samples from twelve species (Fig. 3.74). Two outgroups, *Luscinia luscinia* and *Ficedula parva* are used in the neighbor-joining tree. *Oenanthe hispanica* is seen as a sister species to *Oenanthe pleschanka.* One *O. pleschanka* haplotype was seen to be paraphyletic to *O. hispanica*.

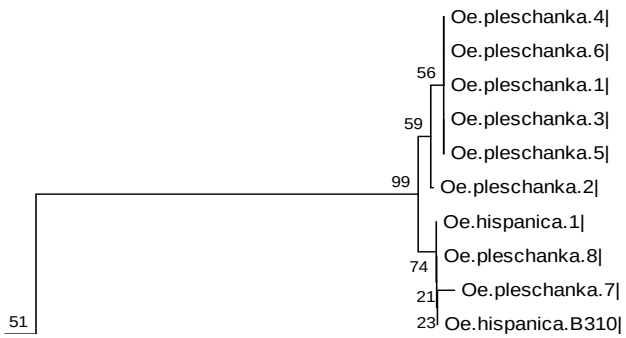

Figure 3.74. Neighbor-joining tree for *Oenanthe hispanica*.

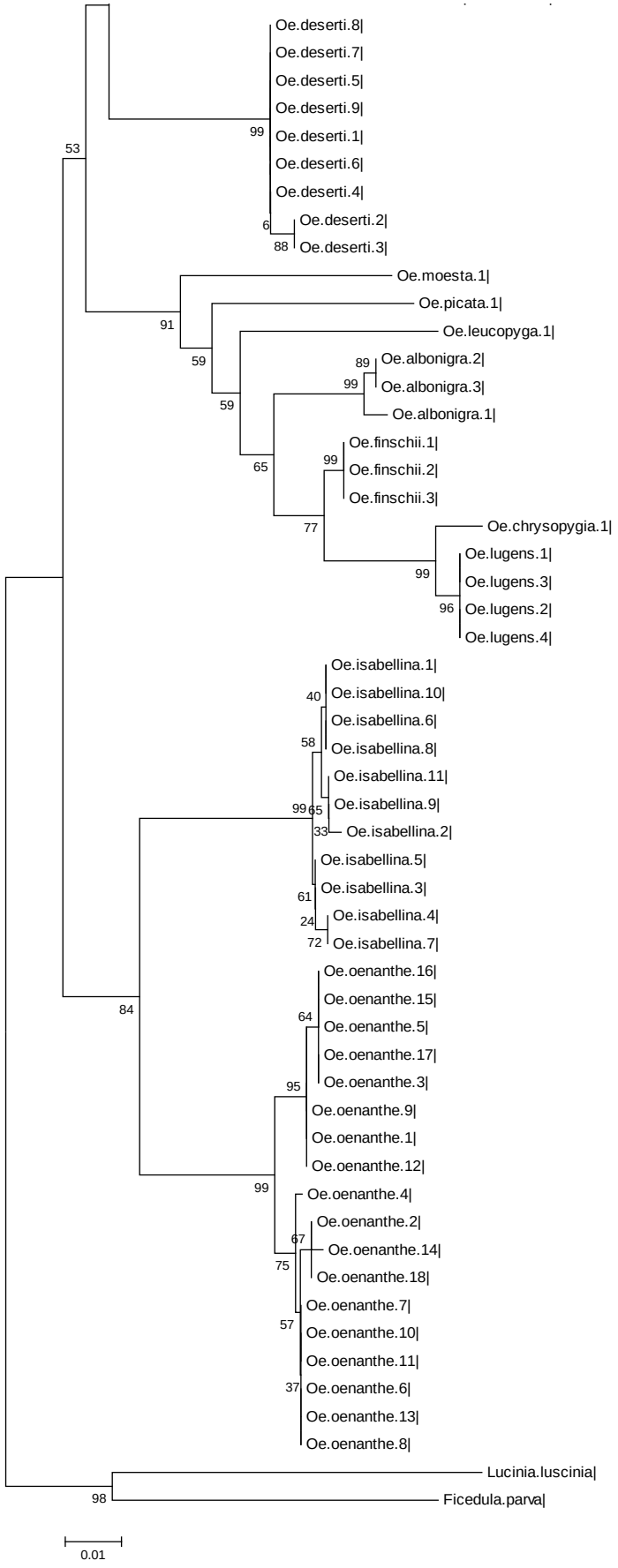

Figure 3.74. continued.

3.23. Genus *Saxicola*

Saxicola rubetra and *Saxicola maurus* are studied from genus Saxicola. Phylogenetic tree contains 27 samples from four species (Fig. 3.75). Two outgroups, *Erithacus rubecula* and *Luscinia megarhynchos* are used in the neighbor joining tree. *Saxicola rubetra* formed a monophyletic clade whereas *Saxicola maurus* showed paraphyly.

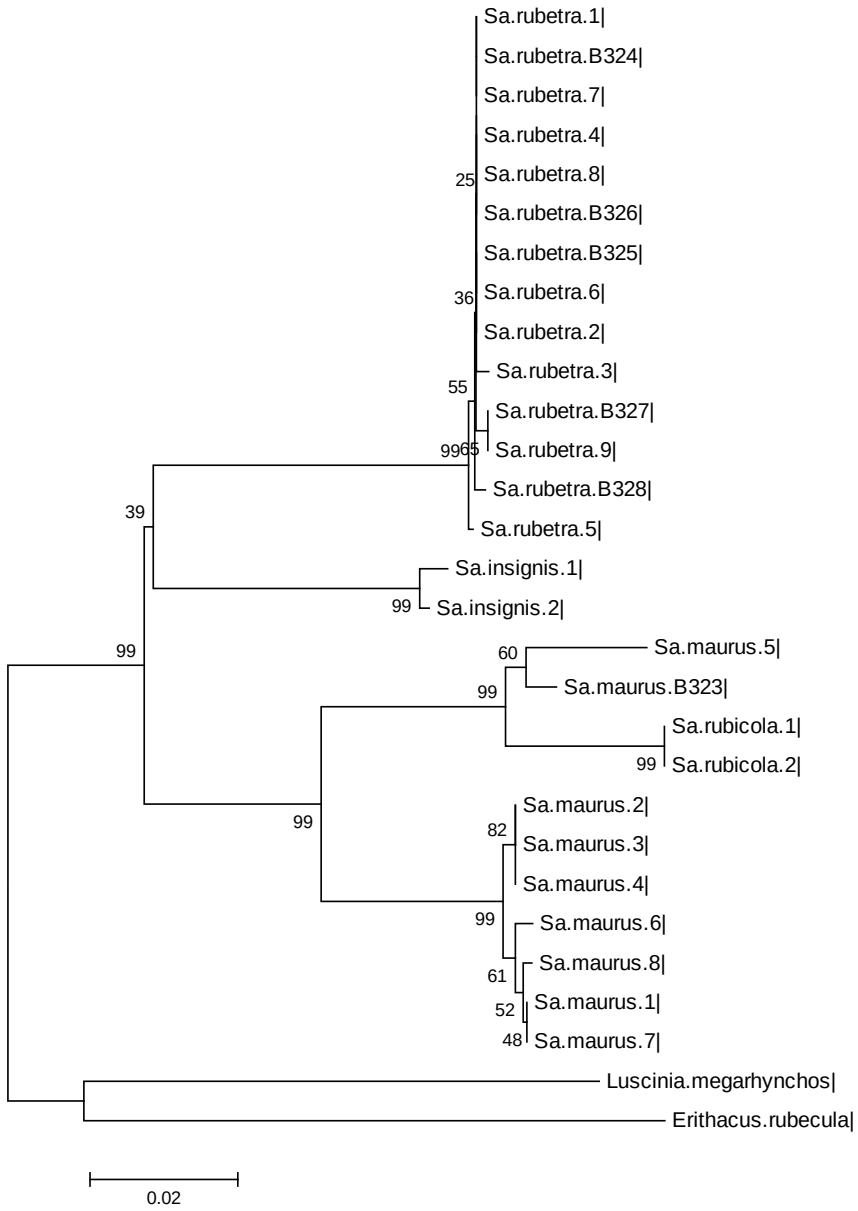

Figure 3.75. Neighbor joining tree for *Saxicola*.

3.23.1. *Saxicola rubetra* (Linnaeus, 1758)

Fourteen different samples from four different countries (Russia, Turkey, Sweden, and Norway) are analyzed for this species (Fig. 3.76). There are five samples collected from Iğdır.

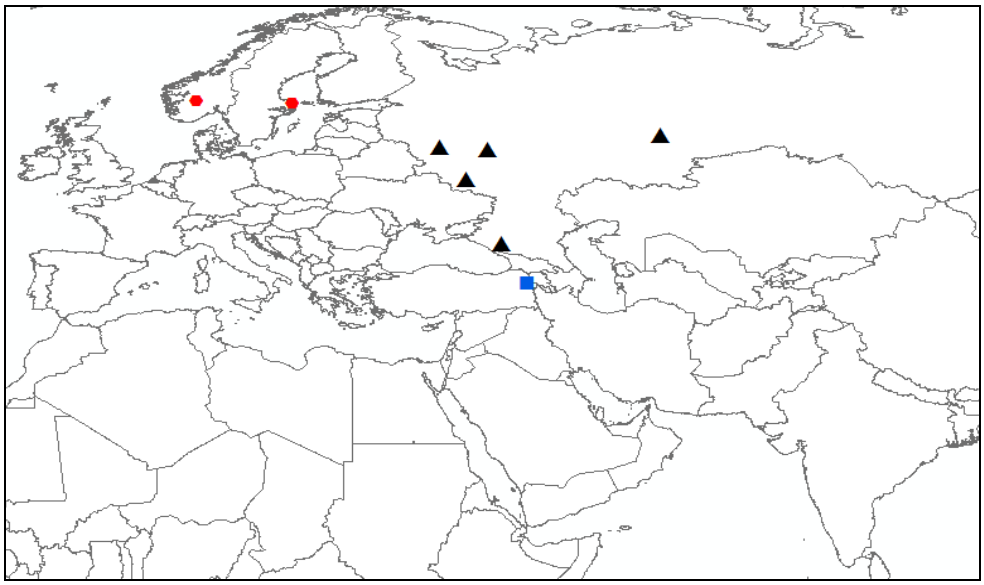

Figure 3.76. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first and second closest matches with Iğdır samples is *Saxicola rubetra* with 100% and 99.84% similarity. Third closest match is *Saxicola insignis* with 92.82% similarity. *Saxicola maurus* is used as an outgroup for the neighbor-joining tree (Fig. 3.77). In the tree, the barcoded individuals from Iğdır (B324, B325, B326, B327 and B328) are clustered closely with the rest of the barcodes from BOLD.

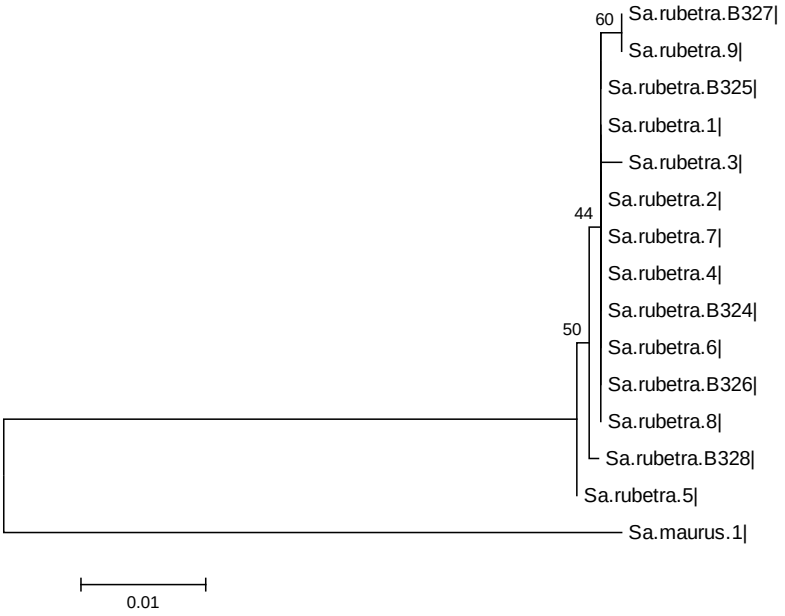

Figure 3.77. Neighbor-joining tree for *Saxicola rubetra*.

There are five haplotypes for this species, which show very low levels of differentiation. Hap 1 is observed in Russia, Turkey, Sweden and Norway, in nine samples. Hap 2 and Hap 4 also are also found in samples from Turkey. Hap 5 is observed only in Russia and Hap 3 is observed only in Sweden (Fig. 3.78).

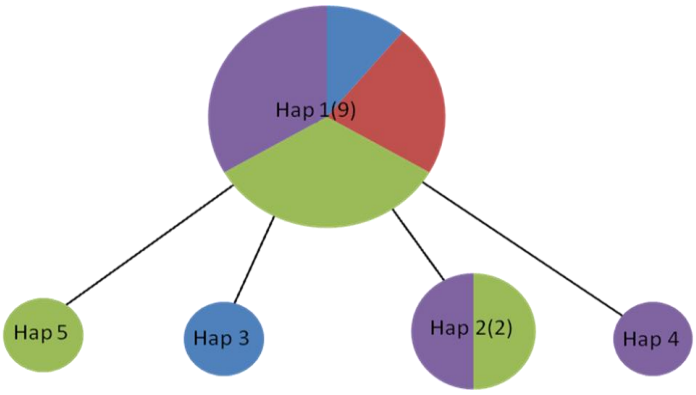

Figure 3.78. Haplotype network for *Saxicola rubetra*.

3.23.2. *Saxicola maurus* (Linnaeus, 1758)

Nine different samples from Russia, Mongolia, Kazakhstan and Turkey are analyzed for this species. There is one sample collected from Iğdır (Fig. 3.79).

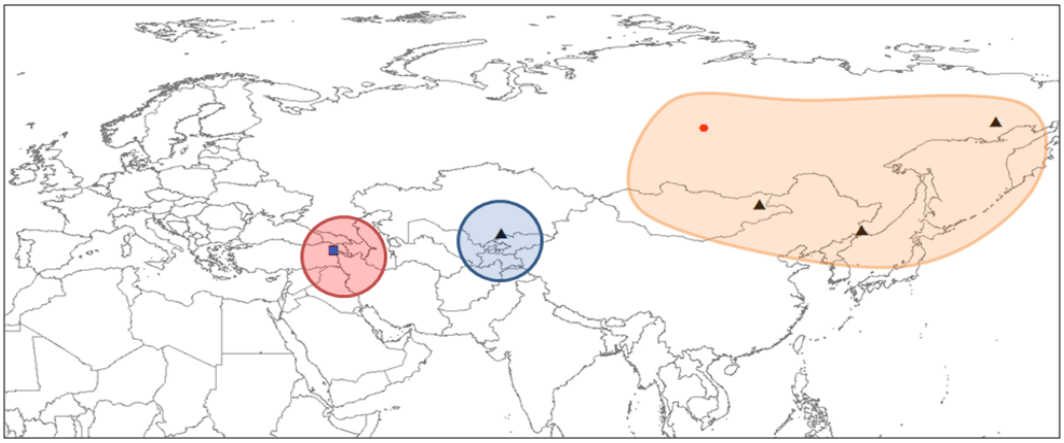

Figure 3.79. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır. Red area indicate Hap 2, blue area indicate Hap 1 and orange area indicate Hap 3, 4, 5 and 6.

A comparison with the BOLD database shows first closest match with Iğdır samples is *Saxicola maurus* with 99.35% similarity. Second closest match is *Saxicola torquatus* with 99.35%. Third closest match is *Saxicola maurus* with 99.19% similarity. *Saxicola rubetra* is used as an outgroup (Fig. 3.80). Iğdır sample (B323) and Kazakhstan sample (*Sa.maurus*.5) formed a separate clade in the neighbor joining tree, apart from the rest of the samples.

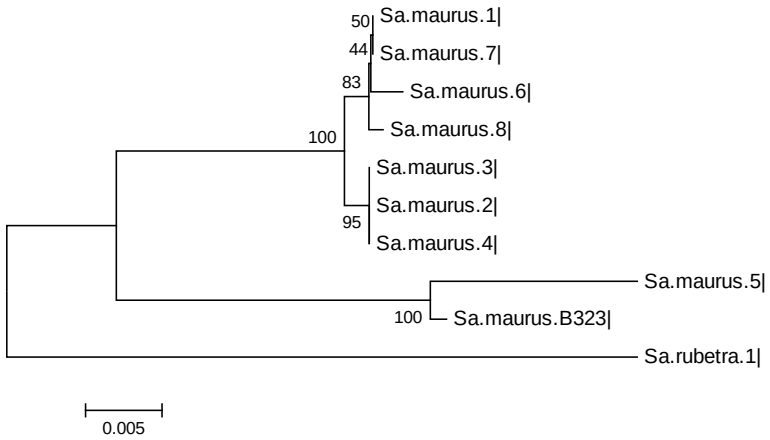

Figure 3.80. Neighbor joining tree for *Saxicola maurus*.

Six different haplotypes are observed for this species. Hap 3, 4, 5 are observed only in Russia in six samples. Hap 6 is observed in Mongolia only in one sample. Hap 2 is observed only in Turkey. Hap 1 is observed in one sample from Kazakhstan (Fig. 3.81).

There are three main haplotype groups observed in the map, one is in Iğdır, the other is in Middle Asia and the last one is in East Asia (Fig. 3.79). It should be noted that Hap 1 and Hap 2 clustered together on the tree (Fig. 3.80), separately from the rest of the haplotypes.

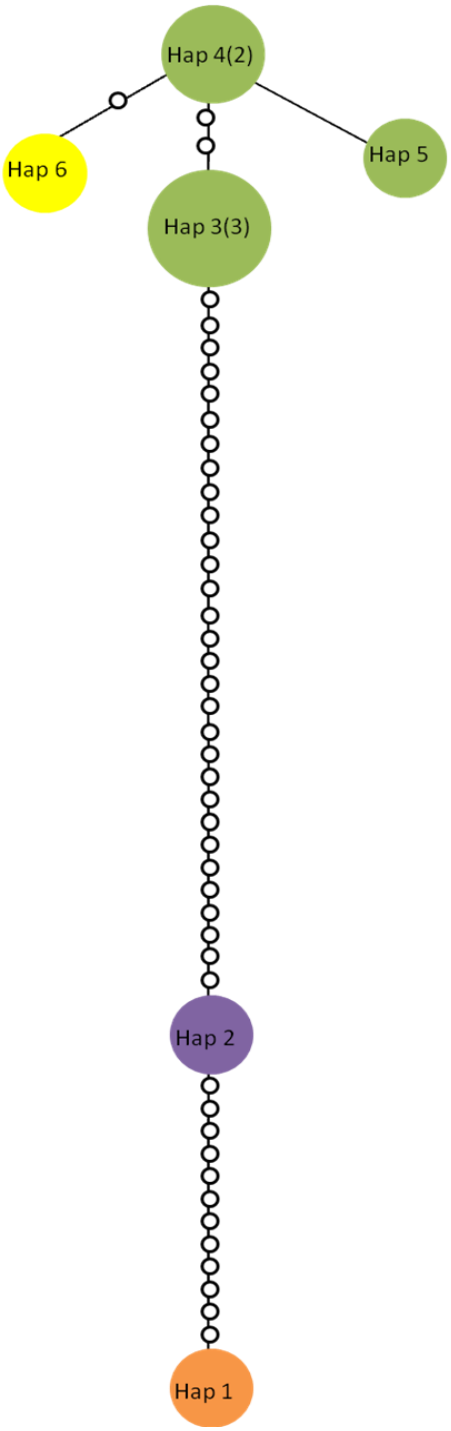

Figure 3.81. Haplotype network for *Saxicola maurus*.

3.24. *Turdus merula* (Linnaeus, 1758)

Fifteen different samples from Russia, Turkey, England, Sweden, and Norway are analyzed for *Turdus merula* (Fig. 3.82). There is one sample collected from Iğdır.

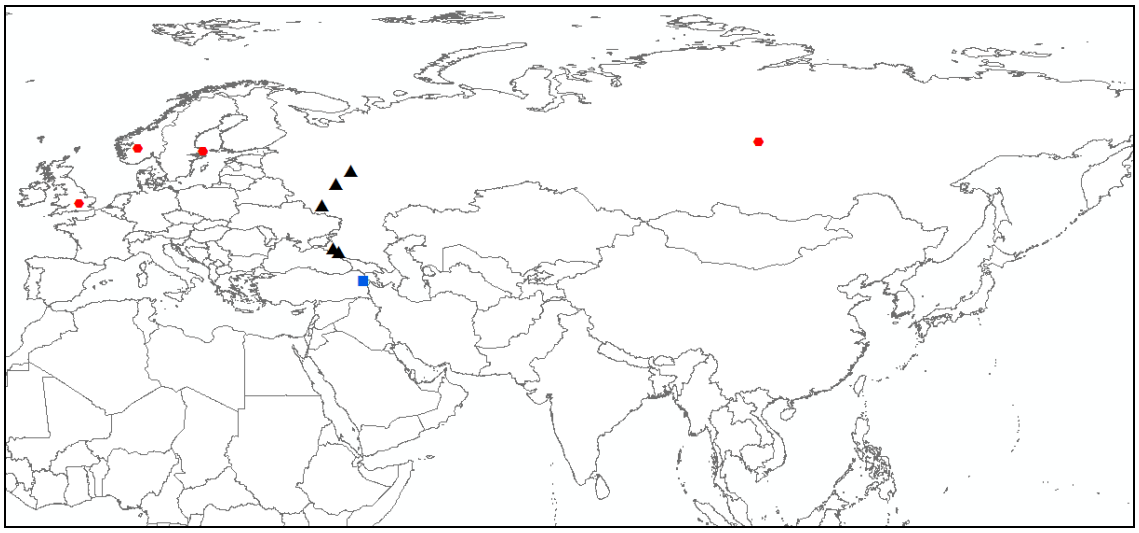

Figure 3.82. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first, second and third closest matches with Iğdır samples is *Turdus merula* with 100% , 99.84% and 99.8% similarity, respectively. Phylogenetic tree contains 141 samples from 22 species (Fig. 3.83). *Sialia mexicana* and *Ixoreus naevius* are used in neighbor-joining tree as outgroups. In the tree, the barcoded individual from Iğdır (B402) clustered closely with the rest of the barcodes of *T. merula* from BOLD.

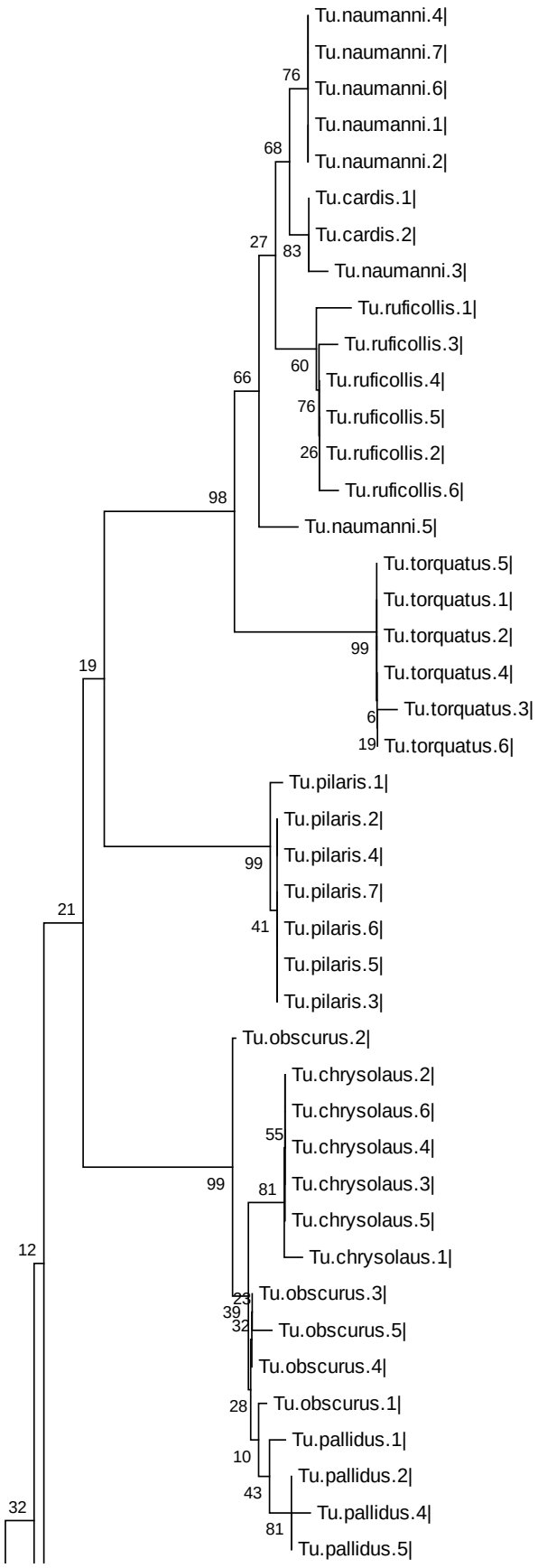

Figure 3.83. Neighbor-joining tree for *Turdus merula*.

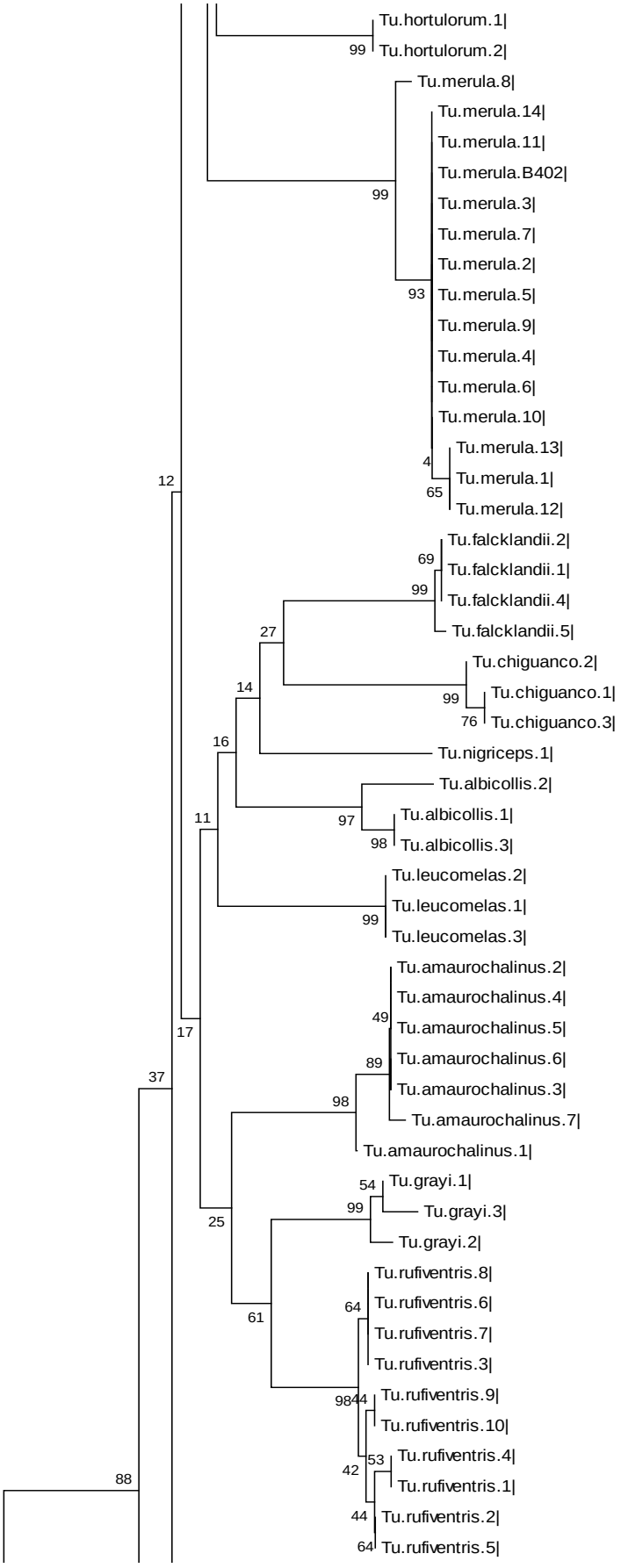

Figure 3.83. continued.

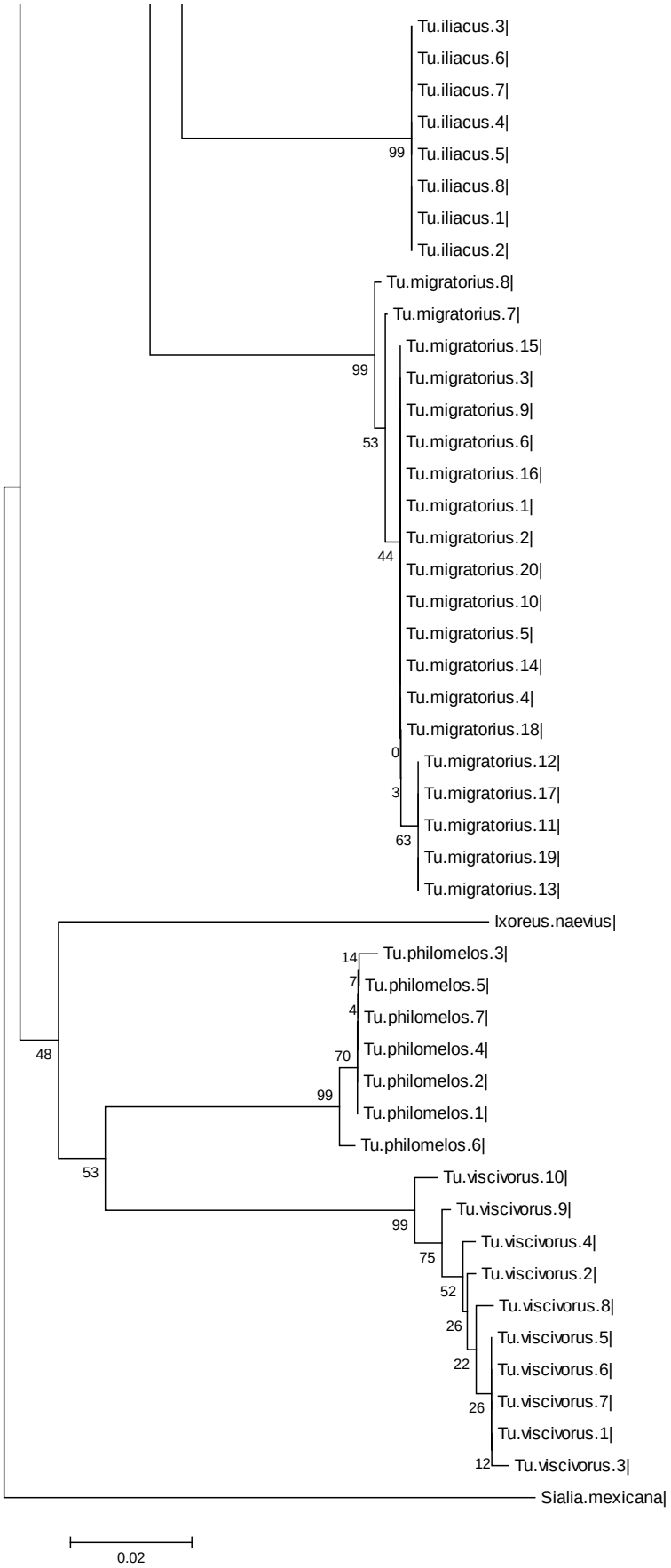

Figure 3.83. continued.

There are five different haplotypes observed for this species, again not with very significant genetic differentiation. Hap 1 is observed in Russia and Sweden, in three samples. Hap 3 is observed Russia, England and Sweden. Hap 2 is observed in Russia, Turkey and Norway. Hap 4 is observed only in Russia (Fig. 3.84).

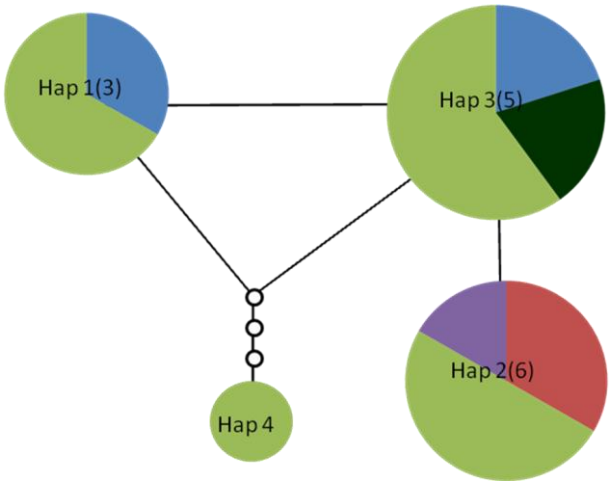

Figure 3.84. Haplotype network for *Turdus merula*.

3.25. Genus *Emberiza*

Four different species, *Emberiza citrinella*, *Emberiza hortulana*, *Emberiza schoeniclus* and *Emberiza calandra* are studied from this genus. A phylogenetic tree made for this genus contains 157 samples and 23 species (Fig. 3.85). Two outgroups *Melospiza georgiana* and *Paroaria capitala* are used in the neighbor-joining tree. As seen in this tree, *Emberiza citrinella* and *Emberiza leucocephalos* do not have unique COI sequences and cluster in one clade. The remaining three species for which we had samples from Iğdır are monophyletic. Species by species details within the genus are given next.

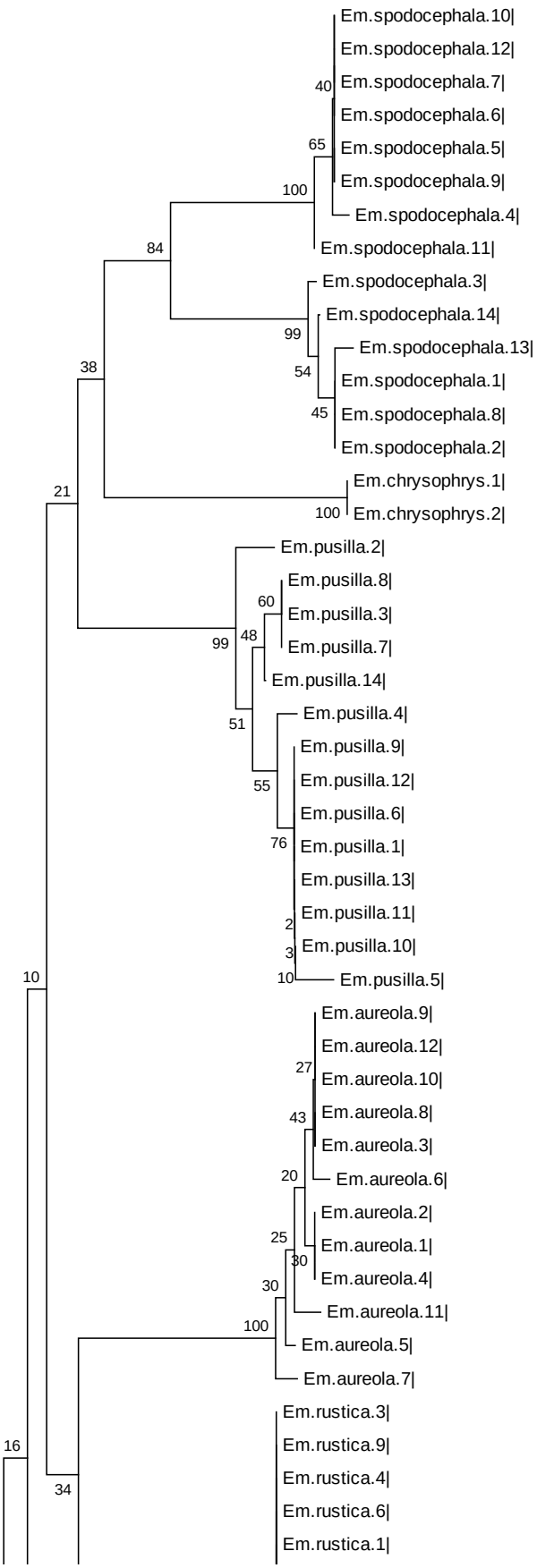

Figure 3.85. Neighbor-joining tree for *Emberiza*.

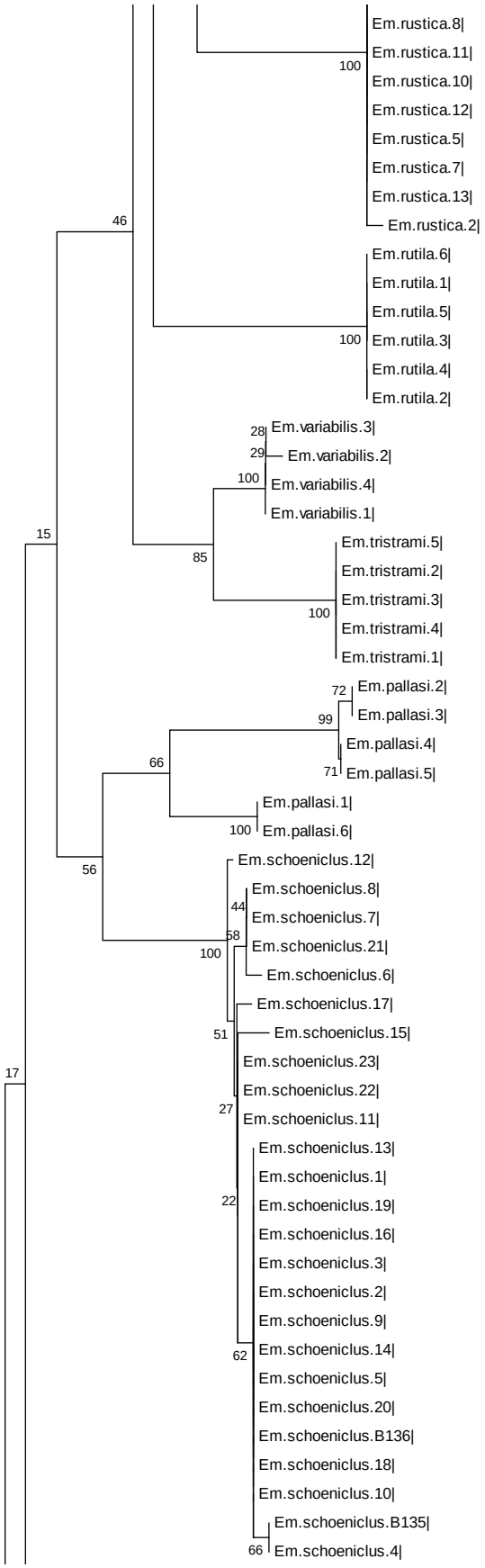

Figure 3.85. continued.

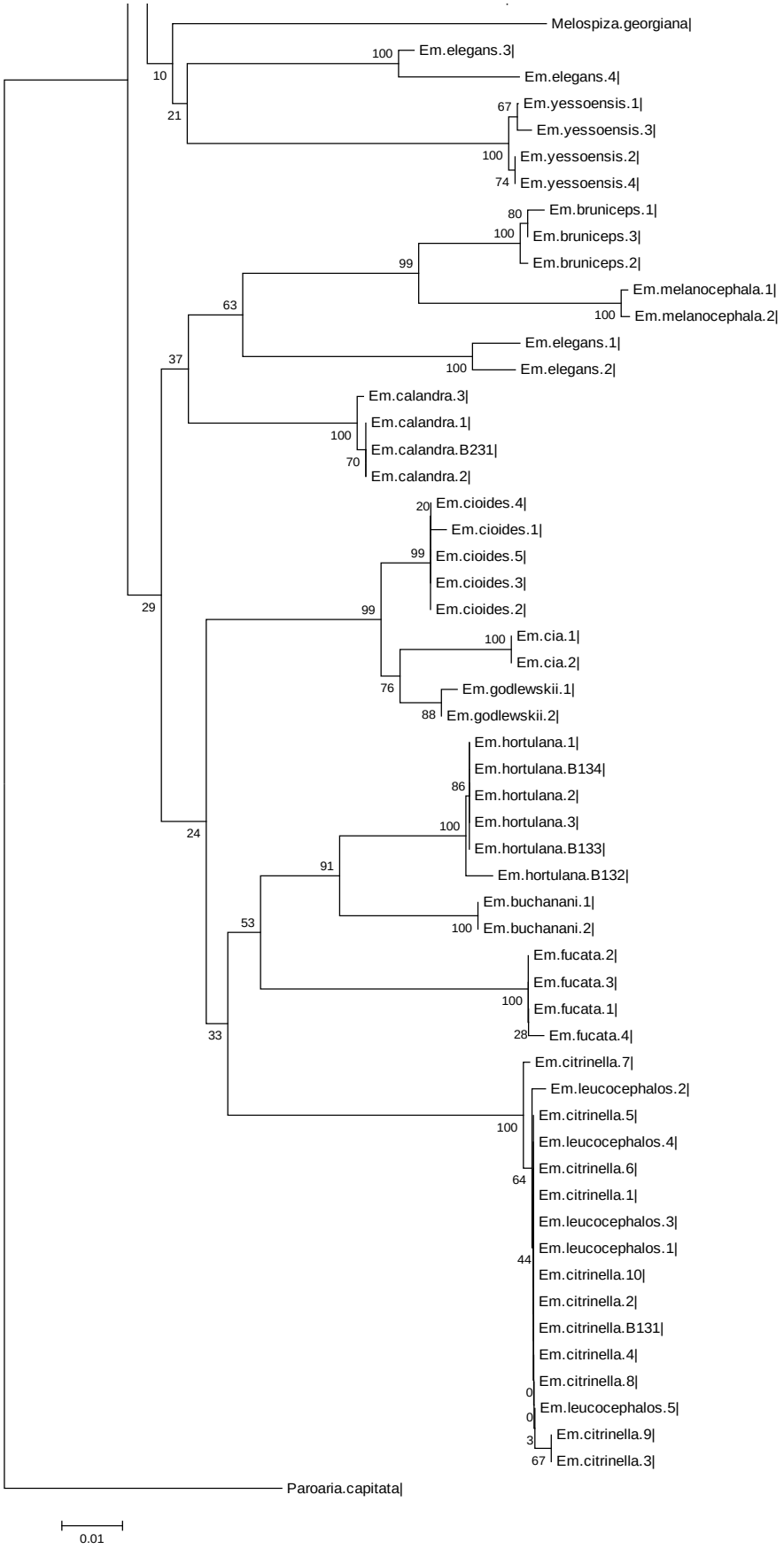

Figure 3.85. continued.

3.25.1. *Emberiza citrinella* (Linnaeus, 1758)

Eleven different samples from four different countries (Russia, Turkey, Norway and Sweden) are analyzed for this species (Fig. 3.86). There is one sample collected from Iğdır.

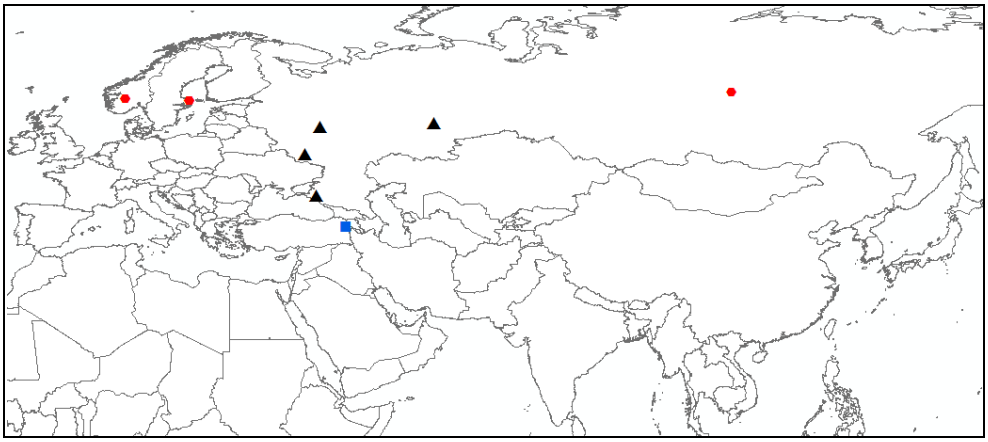

Figure 3.86. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first closest match to Iğdır samples is *Emberiza citrinella* with 100%. This species has % 99.77 similarities with both *Emberiza citrinella* and *Emberiza leucocephalos*. *Emberiza hortulana* is used as an outgroup in the neighbor-joining tree (Fig. 3.87).

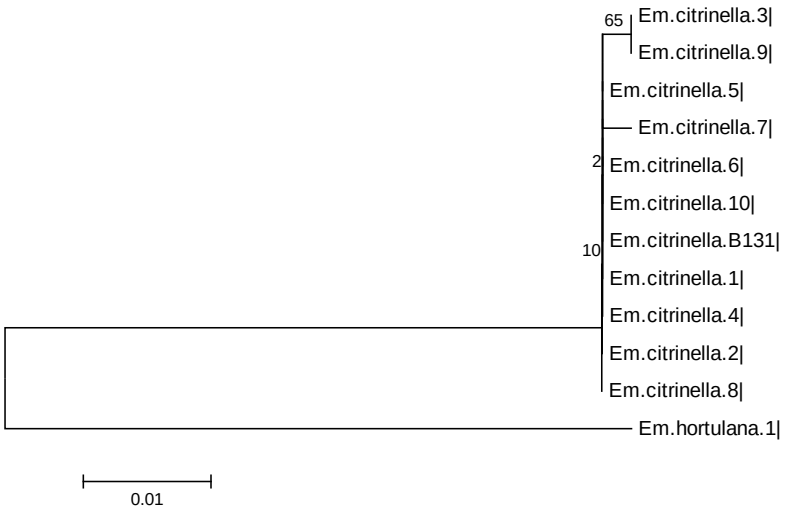

Figure 3.87. Neighbor-joining tree for *Emberiza citrinella*

Three different haplotypes are observed for this species (Fig. 3.88). Hap 2 is the most common, observed in eight samples in four countries, including Turkey. Hap 3 is observed in Sweden and Turkey, and Hap 1 is observed only in Sweden.

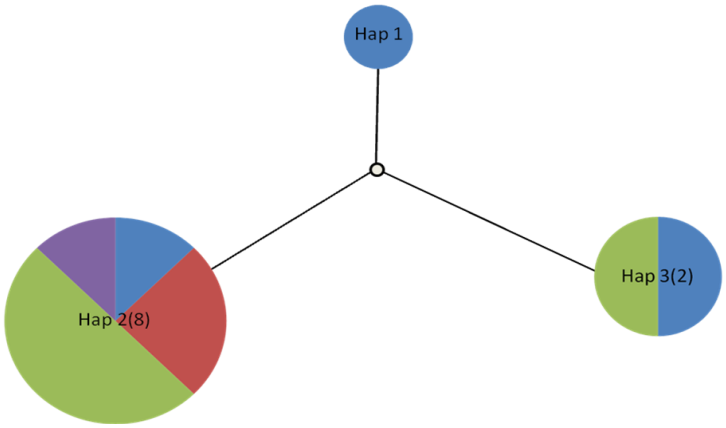

Figure 3.88. Haplotype network for *Emberiza citrinella*.

3.25.2. *Emberiza hortulana* (Linnaeus, 1758)

Six different samples from three different countries (Russia, Turkey, and Norway) are analyzed for this species. Three of six samples are collected from Iğdır (Fig. 3.89).

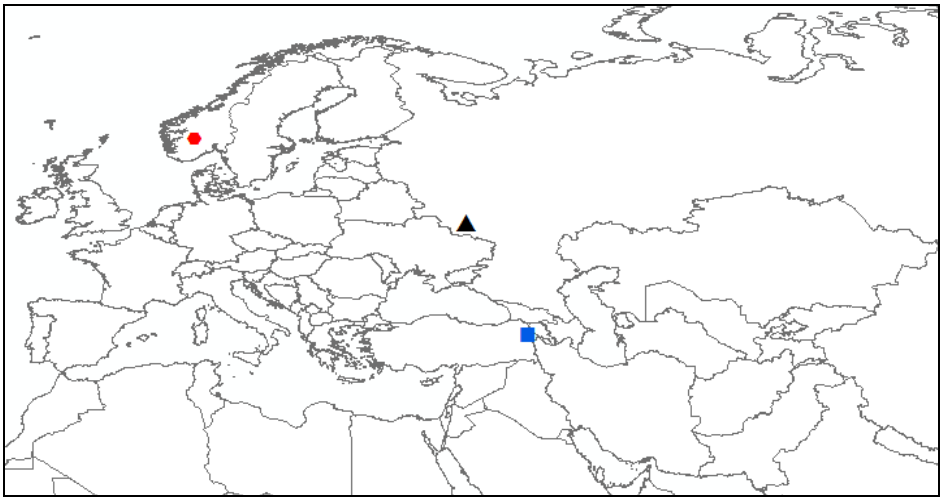

Figure 3.89. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first closest match to Iğdır samples is *Emberiza hortulana* with 99.4% similarity. Second closest match is *Emberiza caesia* with 99.2% and third is *Emberiza buchanani* with 95.21% similarity. *Emberiza citrinella* is used as an outgroup in the neighbor-joining tree (Fig. 3.90). In the tree, *Emberiza hortulana* formed a monophyletic group. Two of Iğdır samples (B133, and B134) are clustered closely in the tree with other samples from BOLD. One of the Iğdır samples (B132) is clustered in a different clade because of three bases difference from Hap 1 that can be seen in the haplotype network (Fig. 4.91). Hap 1 is the most commonly observed haplotype, seen in three different countries, Russia, Norway, and Turkey. For this species there are two new haplotypes recorded for the first time, from Turkey.

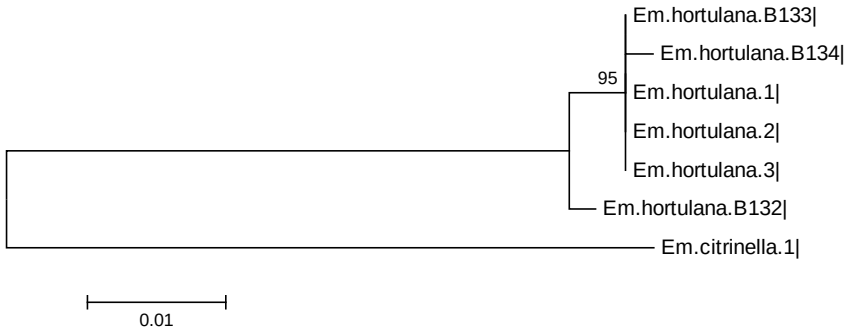

Figure 3.90. Neighbor-joining tree for *Emberiza hortulana*.

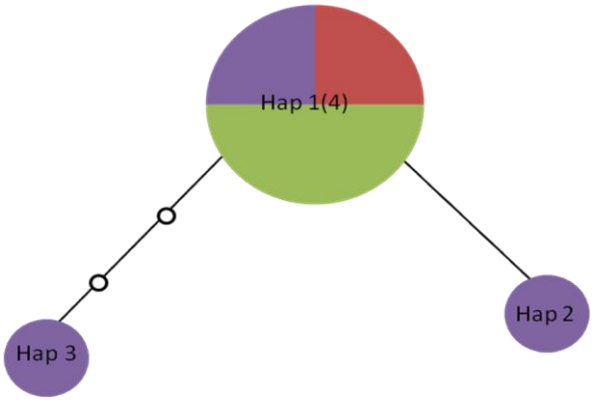

Figure 4.91. Haplotype network for *Emberiza hortulana*.

3.25.3. *Emberiza schoeniclus* (Linnaeus, 1758)

Twenty five different samples from five different countries (Russia, Turkey, Mongolia, Sweden and Norway) are analyzed for *Emberiza schoeniclus*. There are two samples collected from Iğdır (Fig. 3.92).

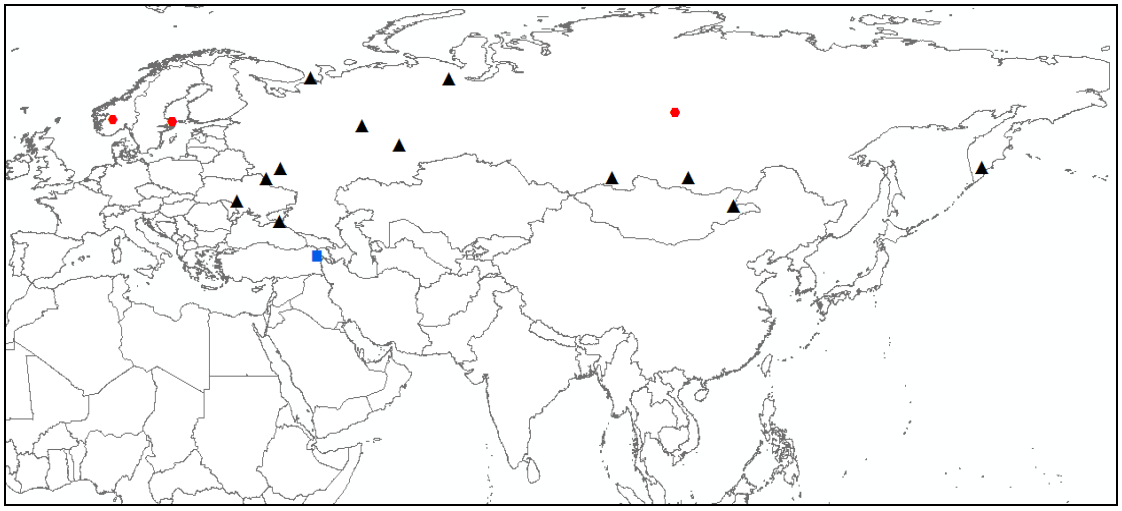

Figure 3.92. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows closest matches are first with 99.84%, second with 99.67% and third with 99.51% similarity to *Emberiza schoeniclus*. *Emberiza citrinella* is used as an outgroup in the neighbor-joining tree (Fig. 3.93). *Emberiza schoeniclus* formed a monophyletic group with samples from Iğdır (B135, B136).

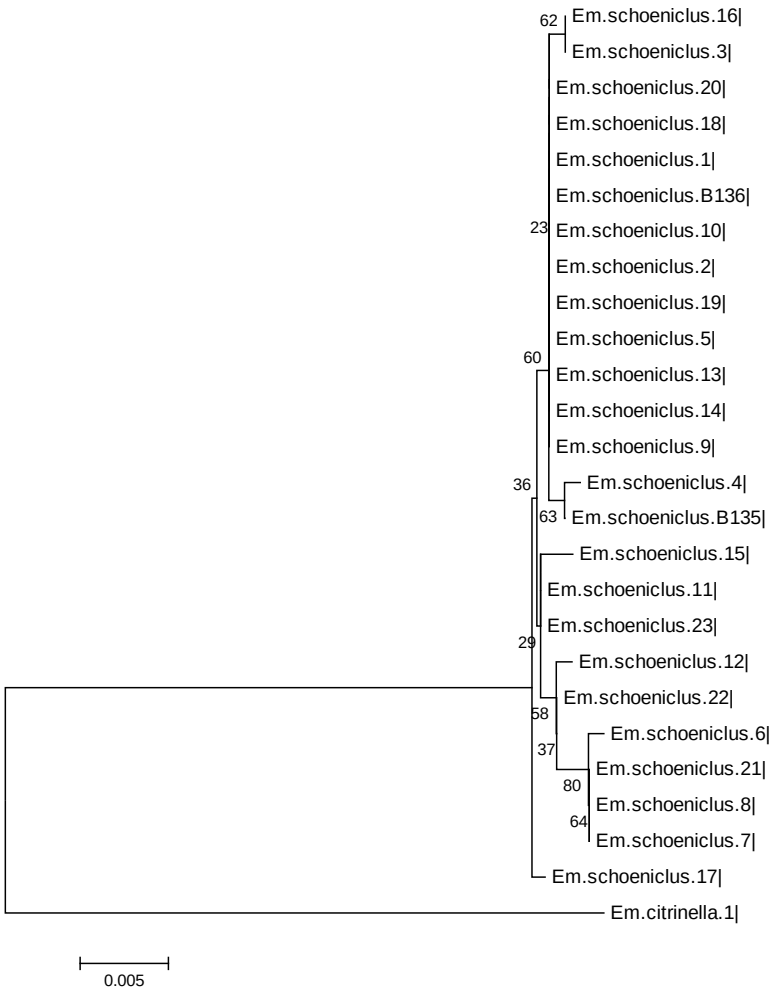

Figure 3.93. Neighbor-joining tree for *Emberiza schoeniclus*.

Eleven different haplotypes are observed for *Emberiza schoeniclus.* Hap 1 is the most commonly seen haplotype observed in eleven different samples from four different countries Russia, Sweden, Turkey and Norway (Fig. 3.94). Hap 2 and Hap 7 are observed in two countries and all remaining haplotypes are observed in one country. Hap 4 is a new haplotype that is observed only in Turkey.

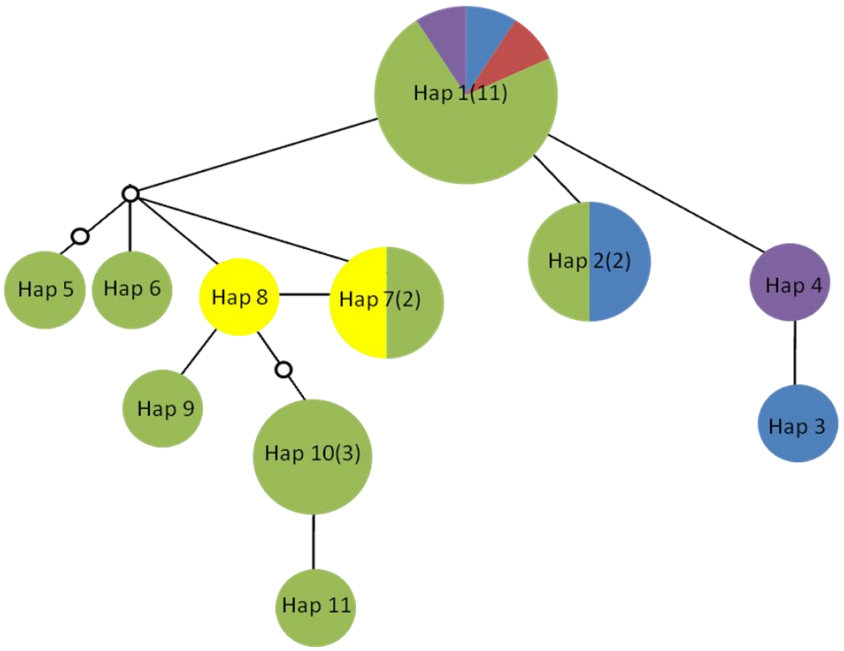

Figure 3.94. Haplotype network for *Emberiza schoeniclus*.

3.25.4. *Emberiza calandra* (Linnaeus, 1758)

Four different samples from three different countries (Russia, Turkey, and Sweden) are analyzed for this species. One sample is collected from Iğdır (Fig. 3.95).

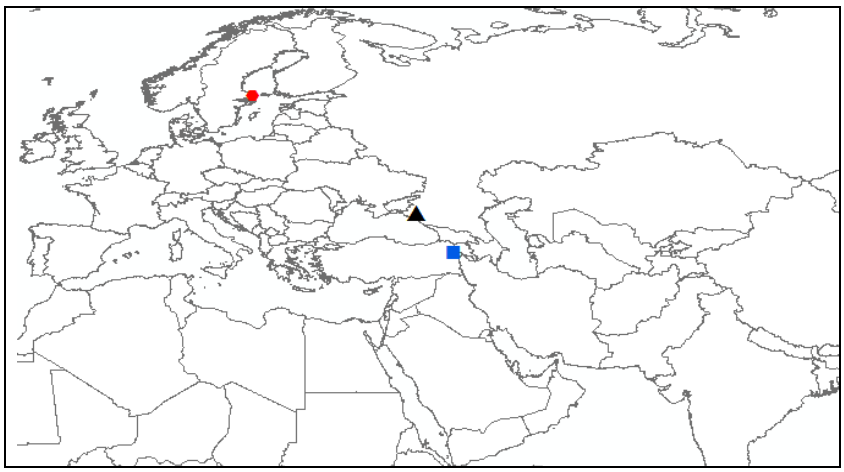

Figure 3.95. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first, second and third closest matches to Iğdır samples is *Emberiza calandra* with 100%, 99.8% and 99.6% respectively. *Emberiza citrinella* is used as an outgroup in the neighbor-joining tree (Fig. 3.96). In the tree the barcoded individual from Iğdır (B231) clustered closely with the rest of the barcodes from BOLD.

Figure 3.96. Neighbor-joining tree for *Emberiza calandra*.

Three different haplotypes are observed in *Emberiza calandra*, which are very little differentiated. Hap 1 is seen both in Turkey and Russia. Hap 2 is observed only in Sweden, whereas Hap 3 observed only in Russia (Fig. 3.97).

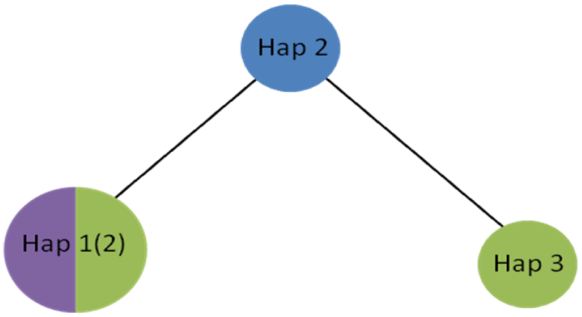

Figure 3.97. Haplotype network for *Emberiza calandra*.

3.26. Genus *Passer*

Two different species, *Passer domesticus* and *Passer montanus* are studied from this genus. Phylogenetic tree contains 55 samples and five species (Fig. 3.98). Two outgroups *Petronia petronia* and *Montifringilla davidiana* are used in the neighbor-joining tree. Both *Passer domesticus* and *Passer montanus* formed monophyletic clades in the tree. *P. domesticus* was a sister clade to *P. hispaniolensis* and both were sister to *P. montanus*.

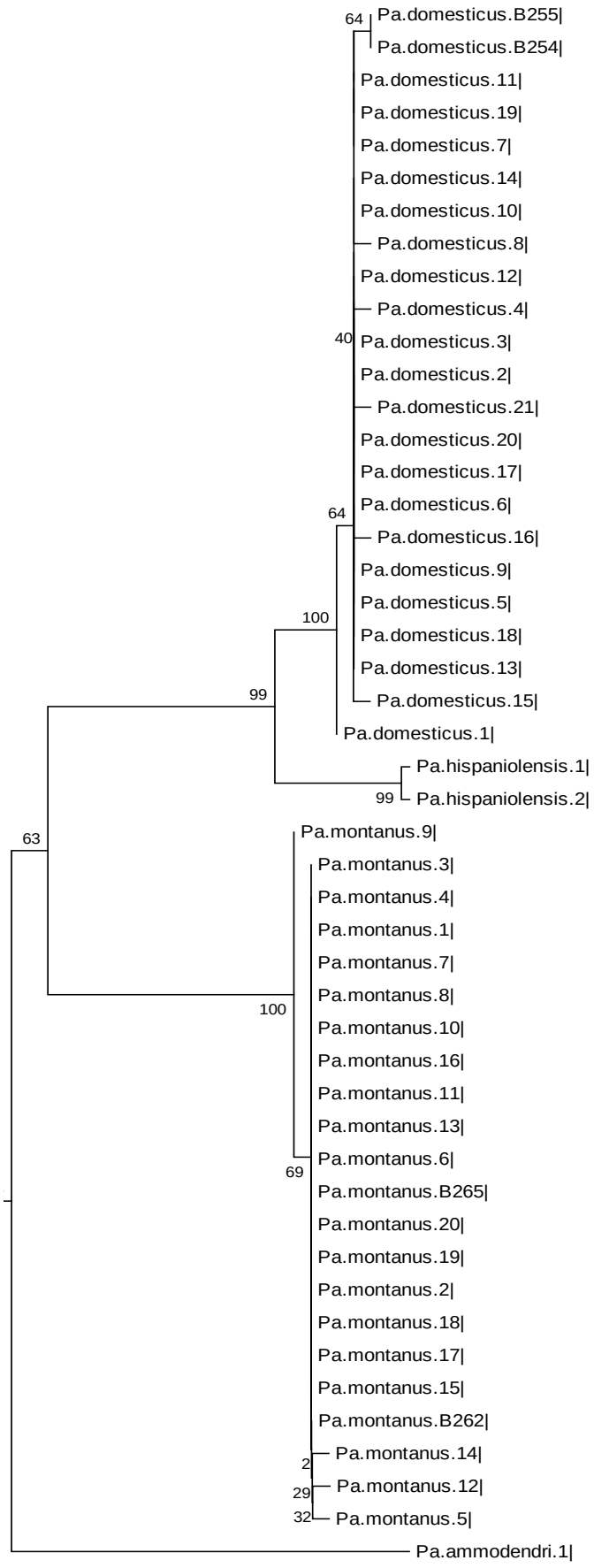

Figure 3.98. Neighbor-joining tree for *Passer*.

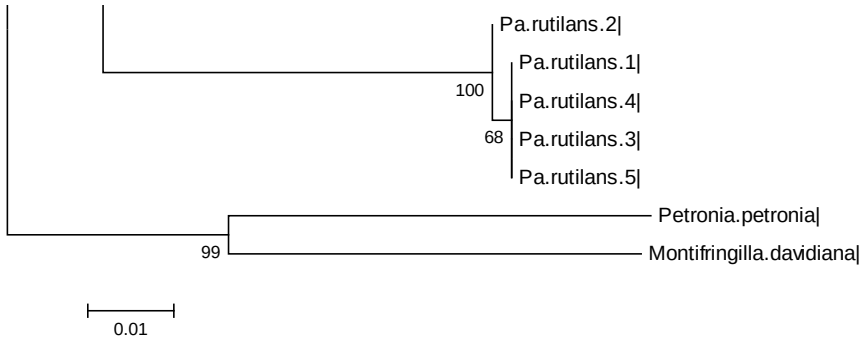

Figure 3.98. continued.

3.26.1. *Passer domesticus* (Linnaeus, 1758)

Twenty three different samples from seven different countries (Russia, Turkey, Argentina, Norway, Kazakhstan, Canada, and Sweden) are analyzed for this species. Two samples are collected from Iğdır (Fig. 3.99).

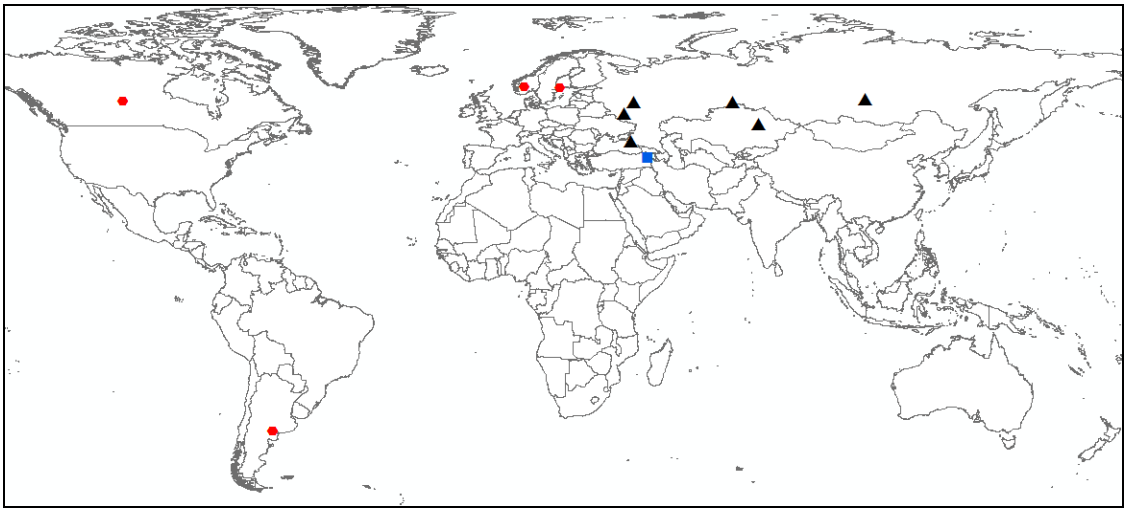

Figure 3.99. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows that first, second and third closest matcher are with 99.79%, 99.58% and 99.37% similarity, respectively, to *Passer domesticus*. *Passer montanus* is used as an outgroup in the neighbor-joining tree (Fig.

3.100). In the tree, the barcoded individuals from Iğdır (B254 and B255) cluster closely with the rest of the barcodes from BOLD from the Palearctic and the New World.

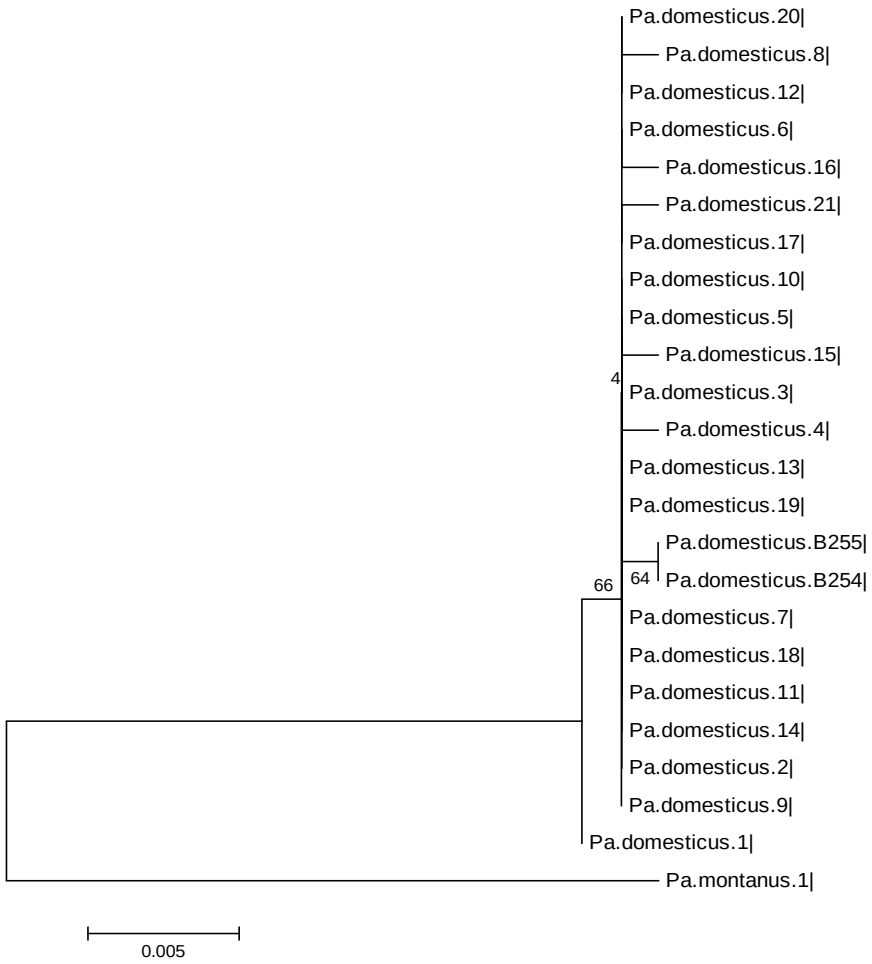

Figure 3.100. Neighbor-joining tree for *Passer domesticus*

Eight different haplotypes are observed for *Passer domesticus*. Hap 1 is the most common haplotype with 15 samples from six countries (Russia, Sweden, Canada, Norway, Mongolia, and Argentina). Hap 2 is only observed in Turkey (Fig. 3.101). All other six haplotypes are only observed in one country and one sample (two from Russia, two from Canada, one Argentina and one Sweden).

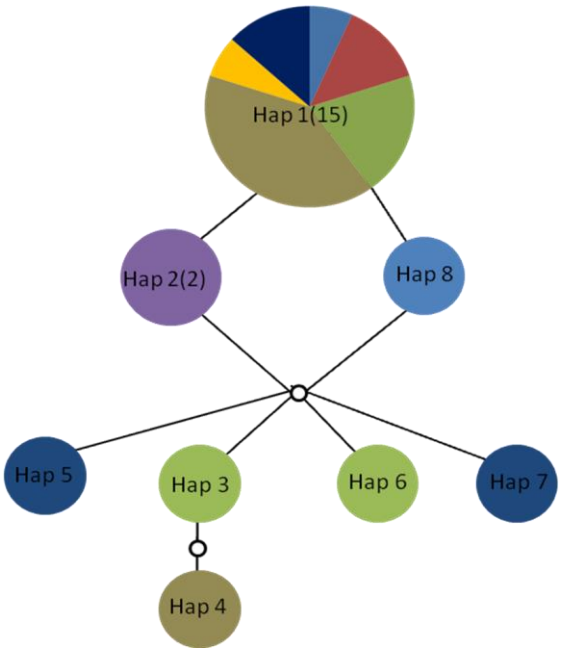

Figure 3.101. Haplotype network for *Passer domesticus*.

3.26.2. *Passer montanus* (Linnaeus, 1758)

Twenty different samples from seven different countries (Russia, Turkey, Mongolia, Norway, Kazakhstan, South Korea, and Sweden) are analyzed for this species. Two samples are collected from Iğdır (Fig. 3.102).

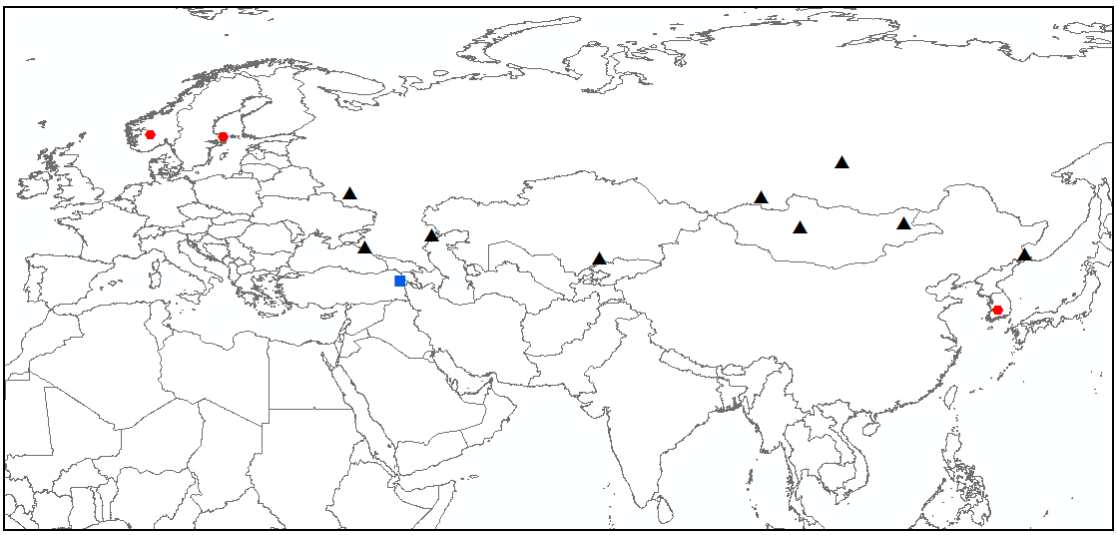

Figure 3.102. The locations for which COI Barcode Data were available from BOLD. The black triangles indicate localities with GPS coordinates, the red circles indicate countries for which GPS data was not available, and the blue squares indicate Iğdır.

A comparison with the BOLD database shows first, second and third closest matches are with *Lanius minor*, with 100%, 99.84% and 99.69% similarity, respectively. *Passer domesticus* is used as an outgroup in the neighbor-joining tree (Fig. 3.103). Iğdır samples (B262 and B265) are clustered closely with other *P. montanus* samples obtained from BOLD.

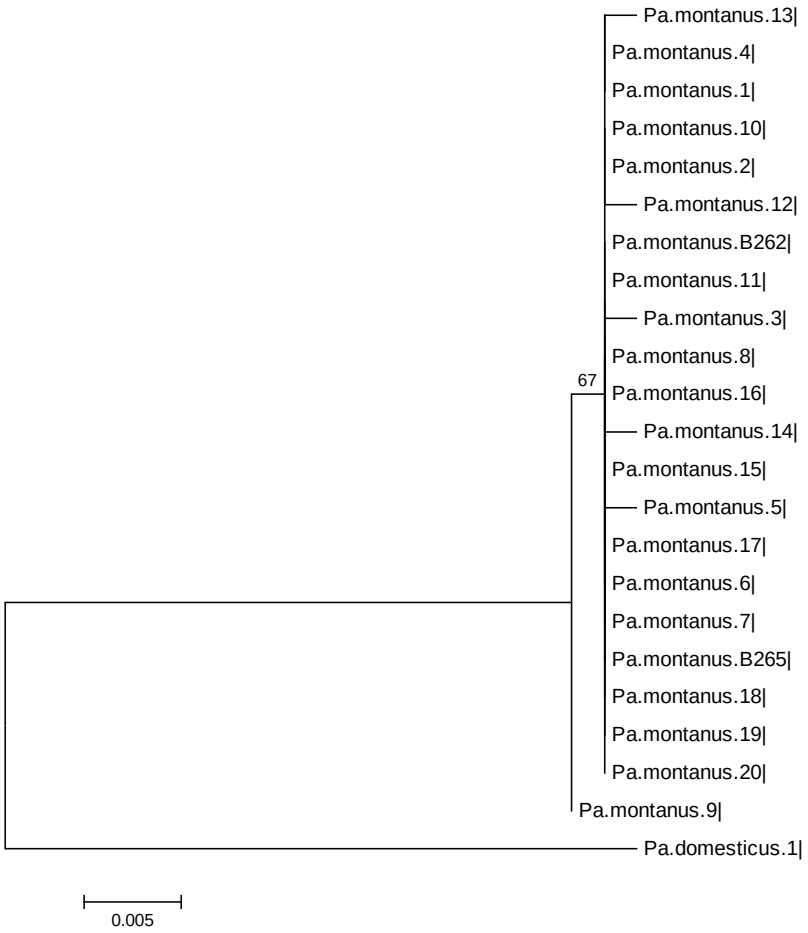

Figure 3.103. Neighbor-joining tree for *Passer montanus*.

Seven different haplotypes are observed for this species, with very little differentiation showing a star-like network. Hap 1 is the most common haplotype seen in 14 samples from seven different countries including South Korea and Sweden (Fig. 3.104). Hap 5, Hap 6 and Hap 7 are observed only in Russia, and Hap 2, Hap 3 and Hap 4 only in South Korea.

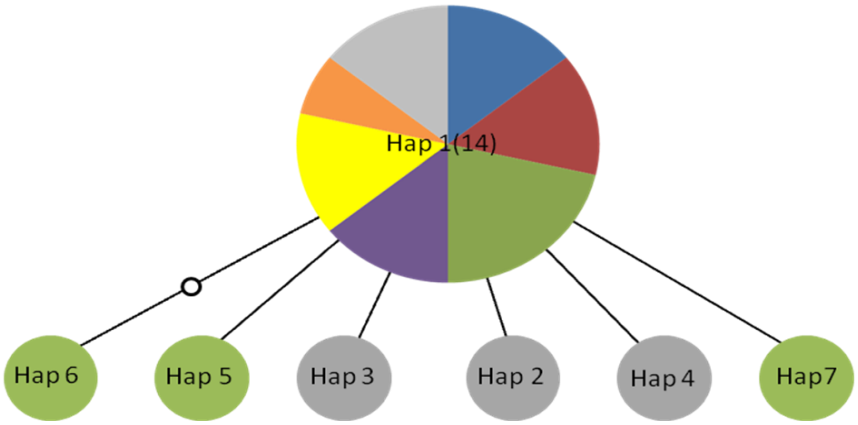

Figure 3.104. Haplotype network for *Passer montanus*.

4. DISCUSSION

The main objective of DNA barcoding is to help with the identification of unknown specimens and improve the discovery of new species. To help in the discovery of a new species or to locate a species, a threshold is needed. This threshold should be high enough to separate specimens that belong to different species and low enough to recognize recently diverged species. Hebert et al. (2004) proposed a threshold to define new species, which he called "barcoding gap", and defined it as ten times the mean intraspecific variation for the studied group. As observed in Fig. 3.1 the mean intraspecific divergence is 0.62% for samples analyzed in this study. So, barcoding gap is 6.2% which is smaller than the smallest interspecific distance of 6.8%. So, for the analyzed species barcoding gap works well.

Samples from five different orders, Passeriformes, Cuculiformes, Galliformes, Coraciiformes, and Caprimulgiformes are studied. In the neighbor-joining tree the Passeriformes and Coraciiformes showed paraphyly. At this taxonomic level, old world warblers and tits were also paraphyletic. It is observed that most of the other families' and genera's positions in the neighbor-joining tree are compatible with the current taxonomy (IUCN, 2011).

From 12 species, 18 new haplotypes are recorded from Iğdır samples. To outline in terms of species, for *Acrocephalus palustris* three new haplotypes are recorded. From *Alcedo atthis*, *Emberiza hortulana* and *Phoenicurus phoenicurus* two new haplotypes are observed. Finally one new haplotype is observed from *Emberiza schoeniclus*, *Ficedula parva*, *Locustella luscinioides, Oriolus oriolus*, *Passer domesticus*, *Phylloscopus trochilus, Saxicola maurus, Saxicola rubetra* and *Sylvia curruca*.

26 of 33 bird species analyzed in this study had unique barcode sequences that are distinct from those found in any other species in the BOLD database. Seven species have shared or overlapping barcode sequences. Four main groups can be formed according to barcoding suitability and intraspecific divergence. First group is composed of five species

(*Acrocephalus palustris*, *Emberiza calandra*, *Lanius minor*, *Merops apiaster*, and *Saxicola rubetra*) which have no subspecies, no intraspecific divergence and have unique DNA barcodes. Second group contains 16 species (*Alcedo atthis*, *Cettia cetti*, *Coracias garrulus*, *Emberiza schoeniclus*, *Galerida cristata*, *Locustella luscinioides*, *Luscinia svecica*, *Muscicapa striata*, *Oriolus oriolus*, *Passer domesticus*, *Passer montanus*, *Phylloscopus trochilus*, *Remiz pendulinus*, *Sylvia atricapilla*, *Sylvia nisoria*, and *Turdus merula*) which have taxonomically defined subspecies, but no intraspecific divergence and have unique DNA barcodes. Third group consists of five species (*Caprimulgus europaeus*, *Erithacus rubecula*, *Parus major*, *Phoenicurus phoenicurus*, and *Sylvia curruca*) which have designated subspecies, have high intraspecific divergence and a DNA barcode that is different from that found in other species. Fourth group is composed of seven species (*Coturnix coturnix*, *Cuculus canorus*, *Emberiza citrinella*, *Emberiza hortulana*, *Ficedula parva*, *Oenanthe hispanica*, and *Saxicola maurus*) which do not have distinctive DNA barcode sequences.

Looking at the patterns in greater detail and starting with the first group, *Acrocephalus palustris*, *Emberiza calandra* and *Saxicola rubetra* had six, three and five haplotypes, respectively. Samples were from Turkey, Russia, Norway and Sweden. Although three new haplotypes are recorded for *Acrocephalus palustris*, all haplotypes were nearly identical. For *Lanius minor* and *Merops apiaster*, Russian and Turkish samples were analyzed. Different haplotypes have only two or three base pair differences for these species. This fact confirms the absence of subspecies in these species, since there was no genetic divergence between samples collected from different locations.

All members of second group have several subspecies, and these subspecies are generally defined according to the differences in morphology like size, bill size or plumage color. For instance, there are seven subspecies recognized for *Alcedo atthis* and samples from Russia, Turkey, Sweden, Norway, Kazakhstan, Mongolia, and South Korea were analyzed in this study. Although European and Korean samples belong to different subspecies, no important intraspecific genetic variance was observed in the DNA barcodes. Other studies also confirm the absence of genetic variability between *Alcedo atthis* subspecies (Moyle et al., 2007). Similarly, there are five recognized subspecies for *Sylvia atricapilla*, seven for *Muscicapa striata*, nine for *Passer montanus*, 11 for *Luscinia*

svecica, 15 for *Turdus merula*, 16 for *Emberiza schoeniclus*, and 37 for *Galerida cristata* (three of these subspecies, *Galerida cristata caucasica*, *Galerida cristata subtaurica*, and *Galerida cristata zion* are observed in Turkey.). Samples from a wide range, including Russia, Turkey, Mongolia, Kazakhstan, South Korea, Sweden and Norway were analyzed for these species. Although these countries cover ranges of many subspecies, all COI sequences were nearly similar. Haplotypes for each species differed by one to eight base pairs. Since subspecies of these species are mainly defined by color or size differentiation, genetic similarity, or lack of genetic differentiation, of these different subspecies is interesting (Voelker et al., 2007).

As another set within the second pattern, *Locustella luscinioides* and *Phylloscopus trochilus* can be given. Each has three recognized subspecies. For *Locustella luscinioides* samples from Turkey and Sweden were analyzed. Samples from Turkey (which are the subspecies *Locustella luscinioides fusca*), were differentiated from Swedish samples (which are *Locustella luscinioides luscinioides*). Similarly the DNA barcode differentiation of *Phylloscopus trochilus* samples we analyzed in this study is in concordance with the accepted subspecies designations. According to our study, samples from both countries had similar DNA barcodes. Similarly, COI sequences for *Passer domesticus* samples from many locations like Russia, Turkey, Argentina, Norway, Kazakhstan, Canada, and Sweden were analyzed. Although 12 subspecies are recognized, a maximum of six base pair differences were observed for this species. Being an introduced species might be the main reasons of similarity in DNA barcodes for the American samples. Although the native range of the house sparrows contains most of Europe and Asia, now this species is found in every continent except Antarctica. Nonetheless, even though both the native and introduced house sparrows have broad phenotypic divergence like body mass, sexual dimorphism and metabolic rate; DNA barcodes analyzed for this study are similar (Drovetski et al., 2004). So, DNA barcode for these species can distinguish *Locustella luscinioides* or *Passer domesticus* from other species but cannot be used for determining subspecies.

Investigating the species in the second group in greater detail, there are three recognized subspecies for *Cettia cetti*. Samples from Russia and Turkey were analyzed and their DNA barcodes were all identical. These samples may belong to *Cettia cetti orientalis*

or *Cettia cetti cetti* subspecies, since this subspecies is observed both in Turkey and Russia. Another species, *Coracias garrulus* has two subspecies. *Coracias garrulus garrulus* is found in North Africa, Europe to Iran and southwest Siberia and *Coracias garrulus semenowi* is found in Iraq to west Xinjiang and south Kazakhstan. Both subspecies winter in South Africa but in distinct locations (Schrey et al., 2011). For this species, samples from Russia, Turkey, Sweden, and Kazakhstan were analyzed. All samples were observed to have nearly identical COI sequences. For these species, it is harder to state the absence of genetic divergence between subspecies, since all analyzed samples may belong to the same subspecies, and there is no previously available genetic data for different subspecies to use as a basis for diagnosis.

A different version of the second group can be recognized for *Oriolus oriolus* and *Sylvia nisoria*. Both these species have two subspecies. One of the subspecies is observed in eastern part of its range from west Siberia to Indian subcontinent, whereas the other in western parts which includes Europe to Ural Mountains. All samples that were analyzed in this study were from western range of these species. Similarly, for *Remiz pendulinus*, all samples analyzed in this study belonged to *Remiz pendulinus pendulinus*, based on their geographical range. So, as can expected, all analyzed DNA barcodes were very similar to each other for this species.

All members of third group have several genetically divergent subspecies and unique DNA barcodes. First members of this group are *Caprimulgus europaeus* with six subspecies and *Sylvia curruca* with two subspecies. For both species two main divergent and geographically isolated haplotype groups were observed. First haplotype group is common in the western parts of the species' range, whereas the second group is common in the eastern parts. So, these species can be called as allopatric population systems. Mean intraspecific divergences were 1.7% and 3.2% for *Caprimulgus europaeus* and *Sylvia curruca*, respectively.

Another member of the third group is *Erithacus rubecula*. All samples except one were similar, although they cover ranges of different subspecies. There are samples from Russia, Sweden, Turkey, and Norway. A genetically different sample was from Russia. Mean intraspecific divergence was 0.9% for this species.

A different pattern in the third group was observed in *Parus major* and *Phoenicurus phoenicurus*. In this study, three and two main different haplotype groups were observed for *Parus major* and *Phoenicurus phoenicurus*, respectively. These different haplotypes had overlapping ranges. Mean intraspecific divergence was 1.7% for *Parus major* and 2.6% for *Phoenicurus phoenicurus*. In this study, most common haplotype of *Parus major* was widely distributed and observed in Sweden, Norway, Russia, Lithuania, and Iğdır. According to Kvist et al. (1999), this fact supports a recent range expansion which can be explained by trapping of this species in a single refuge during the last glaciation period. By the end of this period the species spread to new habitats. For the case of *Phoenicurus phoenicurus*, genetic lineages appear largely sympatric (Kerr et al., 2009), since there is a 26 base pair difference even among the Norwegian samples.

Fourth group is composed of seven species, which are not ideal for DNA barcoding. First members of this group are *Coturnix coturnix* and *Ficedula parva*. Their DNA barcodes are similar with species which were previously accepted as their subspecies. The DNA barcode of *Coturnix coturnix* is 97.94% similar with *Coturnix japonica* and *Ficedula parva's* DNA barcode is 94.52% similar with *Ficedula albicilla*. Although there are still disagreements about recognizing *Coturnix japonica* as a distinct species, it is accepted as a distinct species in this study. By accepting *Coturnix japonica* as a distinct species, no intraspecific divergence was observed in *Coturnix coturnix* species from Russia, Sweden and Turkey. On the other hand, *Ficedula parva* samples had a mean intraspecific divergence of 1.6%, due to the divergent South Korean samples. *Ficedula parva* sample from South Korea is clustered in the *Ficedula albicilla* clade (Fig. 3.41). Since *Ficedula albicilla* is the Asian race and recognized as *Ficedula parva albicilla* by some authorities, it is possible that the South Korean sample that was retrieved from BOLD could be *Ficedula albicilla*, instead of *Ficedula parva*. The non-overlapping distribution and large divergence of the eastern and western haplotypes suggest long-term isolation (Saetre et al., 2001).

Another member of the fourth group, which is not suitable for barcoding, is *Cuculus canorus* since its DNA barcode was 99.53% similar to *Cuculus optatus*. This species also had large intraspecific divergence with a mean of 1.4%. Studies have not determined the cause of the shared mitochondrial haplotypes between these species yet,

and hybrids have never been documented (Gibbs et al., 2000). *Cuculus canorus* and *Cuculus optatus* are taxonomically distinguished by their song differences.

A different pattern in the fourth group is observed in the Emberizidae family and *Oenanthe hispanica*. Sister species from Emberizidae family and *Oenanthe hispanica* are phenotypically distinct but their mitochondrial DNA is similar (Kerr et al., 2009). The DNA barcodes of *Emberiza citrinella* and *Emberiza leucocephalos* are 99.77% similar with each other. These species breed across western and central Siberia, with *Emberiza citrinella* extending to Western Europe and *Emberiza leucocephalos* extending to the Far East. Although two species differ phenotypically, they become more similar across their sympatric area which suggests that they hybridize (Irwin et al., 2009). *Emberiza hortulana* and *Emberiza caesia* are also phenotypically distinct sister species with 99.2% similarity in COI regions. There is no evidence for *Emberiza hortulana* and *Emberiza caesia* to hybridize and their ranges hardly overlap (Alstrom et al., 2008). Studies indicate that the cytochrome-b sequences are more similar in the two sister pairs *Emberiza leucocephalos–Emberiza citrinella* and *Emberiza hortulana–Emberiza caesia* than in other sister species. *Oenanthe hispanica*, which is the sister species of *Oenanthe pleschanka*, had DNA barcodes which were 99.82% similar. It is believed that the main reason of this similarity is their ability to hybridize.

A different version of fourth group is *Saxicola* maurus, with its 99.35% similarity to *Saxicola torquatus*. This species has three different haplotypes which are from three distinct geographical areas, Turkey, Middle Asia and Eastern Asia. Although geographically Turkey is not in the middle, in haplotype network Turkish sample is between Eastern and Middle Asia. These three haplotypes may belong to three different subspecies since ranges of subspecies are consistent with the ranges of haplotypes. Turkish haplotype can be *Saxicola maurus armenicus* since this subspecies is found in the mountains of eastern Turkey to Transcaucasia and Iran. Haplotypes from Central Asia can belong to *Saxicola maurus maurus* since this subspecies is found in East Russia to central Asia; and third haplotype *may be Saxicola maurus stejnegeri* since the range of this subspecies covers East Siberia to Japan and Korea.

In conclusion, genetic barcoding of birds in Kars-Iğdır region gave the similar results with literature both in terms of effectiveness of COI barcodes as identification tools and the existence of a barcoding gap (Herbert et al., 2004). Unique barcodes/haplotypes were seen in 26 of the 33 studied species. Phylogeographic comparisons indicated groups where subspecies designation and genetics matched in some cases and did not match in others. For firmer conclusions more detailed generation of COI sequences is needed for the studied species, both globally and from the remaining parts of Turkey.

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