Convergence in Passeriformes species of Kabul province using COX1 mitochondrial gene sequence

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DOI: https://doi.org/10.22271/chemi.2020.v8.i1ai.8615

Abstract

The aim of this survey is study of Passeriformes of Kabul (Afghanistan). Until now it was thought that these birds are monophyletic. However, recent studies determined that many families of Passeriformes are not monophyletic in traditional classification and extensive molecular investigations are the only way to clarify the phylogeny of this group of birds. Sampling was performed from ten stations in the province of Kabul: ten green, Bagram, Surobi, KhakJabbar, Gol dare, Paghman, Char Asib, Anderabei and Kafurshy, in three stages between 2015 and 2016, 190 samples were captured using Mist net, and 148 samples were transported to the laboratory. 110 samples of them were belonged to Passeriformes and 38 samples were not. Sparrows that were caught were from 13 families, 23 genera and 35 species of Passeriformes, that family Emberizidae with seven species has the highest diversity and family Passeridae with 45 individuals has the highest frequency. After morphometric measurements, the samples were transported to the laboratory for molecular studies, DNA was extracted and COX1 gene was sequenced for identification of species and genetic distances. The Bayesian tree indicated the position of species and genera within families and super families. In this study, genus Passer with four species, genus Motacilla with only one species, genus Carduelis with single species, genus Emberiza with six species and genus Serinus with single species were positioned in superfamily Passerioidea. Genus Corvus with a single species and genus Lanius with one species were located in clade Corvida. Two species of genus Luscinia, one species of genus Muscicapa and one species of genus Acriderobres were located in superfamily Muscicapoidea. Two species of the genus Hirundo, one species of genus Phylloscopus, one species of genus Riparia, one species of the genus Sitta, one species of genus Sylvia and one species of genus Eremophila were positioned in superfamily Sylviaeidae.

Keywords: Convergence, Passeriformes, COX1 mitochondrial gene sequence, Kabul, Afghanistan

Introduction

Accurate identification of wildlife of a country, especially of birds, is a good decision-making criterion for conservation. Therefore, Kabul province, with its good location so far, most of the wild species, especially birds related to sparrows, remain unknown and no complete classification has been made. Kabul Province covers an area of about 3128 square kilometers, equal to one-half percent of the total area of the country (Nuristani, 1972) [28]. With dry weather, Kabul province has an average temperature of 40 degrees Celsius above zero and 17 degrees Celsius, with an average rainfall of 100 to 150 mm per year. The purpose of this study was to obtain the closest distances and closest clusters for evolutionary models, branch lengths and topologies. Bayesian analysis is a statistical method that uses an optimality criterion based on the trait state, except that it does not search for the best tree. In fact, in this method it draws a tree with maximum probability based on information such as evolutionary model, branch length and topology and using the concept of maximum probability. Bayesian analysis, similar to the maximum likelihood analysis of trees, searches for a series of acceptable trees (Roderic and Edward, 1998) [32]. Bayesian analysis is applicable to molecular phylogeny, species phylogeny, and species divergence. This method uses a phylogenetic tree and a model based on the expected value of the parameters and their maximum likelihood, to obtain a posterior probability distribution for those parameters. The model is a tree with a specific topology, specific branch length, and a specific spatial pattern of DNA replacement, and has a specific
distribution rate among nucleotide loci (Philippe et al., 2009).

Materials and Methods
The three methods, mentioned above, are discrete clustering methods. In general, the method selects the most optimal tree or trees with the least evolutionary variations. The method selects the most likely tree or trees, which is more likely than the whole tree. Bayesian analysis performs the search by applying the maximum concept and targeting the probability distribution of phylogenetic trees (Hall, 2008).

Sampled areas
Sampling was carried out in the Kabul province (the capital of Afghanistan) during two consecutive years (2015, and 2016,) in seven sampling sites as shown in Fig 1.6 and seven cities in the province of Kabul, including Baghrami, Sorobi, khaki Jabbar, Shakardareh, Guldareh and Paghman. This sampling included the collection of full and muscle for molecular studies, from the sample the species of this order and a number of other types of orders, a total of about 190 samples. Sampling stations are shown on the map of the province of Kabul with a red mark (Fig. 1).

Table 1: Sampling areas with sampling dates and geographical location

| Altitude | Latitude  | longitude  | Sampling date | Sampling number | Sampling area |
|----------|-----------|------------|---------------|-----------------|---------------|
| 1917m    | -         | -          | 2016          | 4               | Andrabi       |
| 1940m    | -         | -          | 2016          | 2               | Kafroshi      |
| 1748m    | 69°14'54.44"E | 34°38'54.06"N | 2015          | 5               | Dehsabz       |
| 1748m    | 69°14'54.44"E | 34°38'54.06"N | 2015          | 6               |               |
| 1748m    | 69°14'54.44"E | 34°38'54.06"N | 2015          | 35              |               |
| 1748m    | 69°14'54.44"E | 34°38'54.06"N | 2016          | 22              |               |
| 1791m    | 69°16'31.61"E | 34°28'63.32"N | 2015          | 4               | Bagrami       |
| 1791m    | 69°16'31.61"E | 34°28'63.32"N | 2016          | 1               |               |
| 1001m    | 69°44'48.94"E | 34°35'18.09"N | 2015          | 5               | Serobi        |
| 1001m    | 69°44'50.53"E | 34°35'19.42"N | 2016          | 5               |               |
| 1001m    | 69°44'48.94"E | 34°35'18.09"N | 2016          | 5               |               |
| 2309m    | 69°26'00.09"E | 34°20'00.02"N | 2016          | 3               | KhakieJabar   |
| 2337m    | 69°66'51.91"E | 34°24'57.55"N | 2016          | 5               | ChaarAsiab    |
| 2208m    | 69°58'41.62"E | 34°35'05.24"N | 2016          | 20              | Paghman       |
| 1991m    | 69°02'24.70"E | 34°44'41.94"N | 2016          | 9               | Guldareh      |
| 1991m    | 69°02'24.70"E | 34°44'41.94"N | 2016          | 4               |               |
| 1994m    | 69°04'18.87"E | 34°41'51.09"N | 2015          | 4               | Shakardara    |
Sampling method
In different habitats, the Misnet was used for catching samples, and by singing the sound of the expected species in each habitat. After catching the bird (Fig. 2.1.2), it was removed from the net, and photographs were taken from the back, abdomen and lateral surfaces before changing the feathers. Then separate a few samples (such as those from the primary and secondary master bird) and put in a zipper bag. The sample number, the specifications of the sampling site (name of the location, the geographical location) and the sex of the bird were recorded on the envelope. Then, from the chest and muscle of the bird (Fig. 2.1), the tissue samples were taken into vials containing alcohol, and the vials were numbered.

Sampling equipment
The equipment used in this study were Misnet (for catching), GPS for geographical location, binocular, camera, alcohol, containing, containers, vials, envelope, digital caliper, plaster bags, paper, permanent marker. Invisible net (Misnet) for tracking GPS bird to measure longitude and latitude of area 3 Map of the area, binoculars, cameras, containers containing alcohol to store dead specimens, vials containing alcohol, to fix muscle tissue, envelope, to hold the full digital caliper for measuring morphological traits, anatomical instruments for removing tissue, plastic box or paper for storing vials, padding for measuring wings, markers.

Molecular studies
For molecular studies, tissues and bird feathers were used.

(a). Separating and storing tissues for molecular studies
The tissues were kept in alcohol. The tissue size was not too large because the water in the tissue dilutes the alcohols were replaced after a few hours. Different tissues such as muscle, liver and kidney can be used to extract DNA but the molecular tissues were used in this study. The tissue parts were separated from the tissue samples in a completely sterile condition and placed in another vial and the sample number was recorded. The parts are better in size and small in size.

DNA extraction
Necessary material for DNA Extraction
1. Extraction buffer (µL140)
2. Proteinase k (µL10)
3. Sterile water (µL50)
4. NaCl (µL180)
5. 10% SDS (µL80)
6. Isopropanol (µL420)
7. Ethanol 80% (2 x 250 µL)

DNA extraction steps
10 µl Proteinase K, 80 µl SDS 10%, and 410 µl Extraction Buffer were added to the sample containing vial, and were placed in 37c temperature for 12-244 in a _____. After that, the sample were centrifuged at about 13000 rpm for 5 minutes and the supernatant was transferred to a new sterile vial. After adding 180 µl of NaCl to vial, it was again centrifuged for 5 minutes at 13,000 rpm. The supernatant was poured into a new sterile vial and added 420 microliters of cold isopropanol to each vial. After mixing the contents of the vial, they were centrifuged for 5 minutes at 13000 rpm and the supernatant was discarded. The contents of the vial were washed with alcohol in two steps and, at each washing step, 250 µl of 80% ethanol was added to each vial and centrifuged for 5 minutes at about 13000 rpm. In the final wash step, in order to completely eliminate the effect of alcohol, they can be placed in a vacuum or in the absence of a vacuum, the vials were left open and placed in the hood for several hours to evaporate the alcohol. After assuring evaporation of alcohol, 50 µl of distilled water were added and mixed. After that, the extracted DNA can be stored for a long time at -20 ° C.

Quantifying the quality and quantity of DNA
The quantity and quality of extracted DNA are measured using a Nanodrop device or electrophoresis gel. In the measurement with a Nano drop device, the device is calibrated with the solution used for diluting the DNA (here were distilled water), and then the amount of 1 microliter of extracted DNA was placed on the sensitive surface of the device and the genome was studied quantitively and quantitively (Fig. 2).

![Fig 2: Diagram of DNA Acquiring in Nano drop System.](http://www.chemijournal.com)

By using nano drop, the amount of DNA present in the cell is expressed in ng/µl, as shown in Figure 2-2, the DNA value in the sample is equal to 2.123 ng/µl. The quality of the extracted DNA is calculated based on the wavelength ratio A260/A280, and it approximates where the ratio is between 1 and 2.

Agarose gel electrophoresis
An electrophoretic gel can also be used to determine the quantity and quality of DNA. The brightness and thickness of the desired bands show good information about the DNA at the extraction stages as well as in the PCR stage. Electrophoresis at the extraction stage can reflect the state of DNA in terms of degeneration and in the PCR stage it can indicate the proliferation of the desired bond or the formation of dimer dimer on the gel.

Preparation of the gel and its buffer
To make the gel, the following procedure was performed: 0.4 g of agarose was dissolved in 50 ml of TBX buffer and placed on microwave to clarify the gel solution.

TBX Buffer Material:
1. TRIS 2. Boric acid 3. EDTA (ethylene diaminetetraacetic acid)
The buffer was prepared initially 5X and converted to 0.5X when used.
To prepare a TBX 5X buffer, 3.44 g boric acid, 6.75 g Tris and 2.5 ml EDTA were mixed and by adding distilled water was reached to a volume of 125 ml. At the time of use, it was diluted 10 times with distilled water and prepared to 0.5X.
Preparing the gel to put the samples
After the mixture of agarose and TBX buffer was cooled slightly, 5 μl of Green Viewers was added to it, and was poured into a gel plates whose tow ends were previously sealed with gel. After the gel was formed, the shoulders were removed and the gel was placed in an electrophoresis tank containing 0.5 X TBX solutions.

Transfer of sink sump well
After adding 4 μl of leading solution to 2 μl of PCR product or extraction of DNA, the sample was placed inside the gel well, and the DNA ladder was put in the first well. Then, due to the negativity of the DNA charge, the product was placed in the negative pole to the positive pole on a gel at a voltage of 90 volts. The time required for electrophoresis is 20-15 minutes.

**See the gel in the gel documenting machine**
The documenting is used to see bends on the gel and the gel is examined under UV light. With this device you can take a gel image.

**Results**
In this chapter, results from the molecular approach were obtained using the gene sequences found in the NCBI and BOLD gene search engines. In this study, 1240 sequences of the gene bank and 86 sequences from 190 samples of birds captured from Kabul province were investigated in a molecular analysis. The bayesian tree was drawn by MrBayes software for all collected samples from Kabul province. In this study, 82 sequences were assessed from which 81 were from Kabul and one was chosen from gene bank as out of genus sample. The tree was divided mainly into two cladets **Corvida** and **Passerida**.

**Identification of samples of passeriformes in Kabul province**
A total of 190 specimens of passeriformes and non-sparse forests were captured during the study in Kabul province in 2015 and 2016. Based on morphological traits and using valid identification keys from existing samples, 148 samples were selected for morphological and molecular study. Among 148 samples, 110 specimens were related to other with 13 families, 23 genera and 35 species. Their scientific names based on family presented in table 1.

| S. No | Scientific name         | Family name | Number of Sp. |
|-------|-------------------------|-------------|---------------|
| 1     | Laniusvittatus          | Laniidae    | 1             |
| 1     | Laniusschach            | Laniidae    | 2             |
| 1     | Golden oriol           | Oriolidae   | 3             |
| 1     | Corvusmonedula         | Corvidae    | 4             |
| 1     | Alaudaevensis           | Alaudidae   | 5             |
| 1     | Eremophilaaparistris    | Alaudidae   | 6             |
| 3     | Calandrellabrachydactyla| Alaudidae   | 8             |
| 2     | Hirundorustica         | Hirundinida | 9             |
| 2     | Phylloscopusnornatus    | Phylloscopida| 10            |
| 12    | Sylvia curruca         | Sylviae     | 11            |
| 1     | Sittatephronota        | Sittidae    | 12            |
| 1     | Acridotherestrists      | Sturnidae   | 13            |
| 1     | Temenuchaspatagorarum   | Sturnidae   | 14            |
| 2     | Luscinaniagaristoch     | Muscicapidae| 15            |
| 2     | Lusciniavecica         | Muscicapidae| 16            |
| 1     | Muscicapastriata       | Muscicapidae| 17            |
| 12    | Passer domesticus      | Passeridae  | 18            |
| 8     | Passer montanus        | Passeridae  | 19            |
| 3     | Passer hispanioleisis  | Passeridae  | 20            |
| 2     | Motacillaflava         | Motacilliida| 21            |
| 2     | Anthus hodgsoni        | Motacilliida| 22            |
| 2     | Anthus pratensis       | Motacilliida| 23            |
| 1     | Calandrellabrachydactyla| Alaudidae   | 24            |
| 1     | acutirostris           | Alaudidae   | 25            |
| 2     | Cardueliscarduelis     | Fringillidae| 26            |
| 3     | Pyrrhalipyrhilula      | Fringillidae| 27            |
| 1     | Serinspasilius         | Fringillidae| 28            |
| 12    | Emberizabaniceps      | Emberizidae | 29            |
| 2     | Emberizahortulana      | Emberizidae | 30            |
| 8     | Emberizabuchanami      | Emberizidae | 31            |
| 1     | Emberizaleucocephalus  | Emberizidae | 32            |
| 1     | Emberizacia            | Emberizidae | 33            |
| 5     | Emberizamelanocephala | Emberizidae | 34            |
| 9     | Emberizastewarti       | Emberizidae | 35            |

**Molecular results of Passeriformes in Kabul province**
A total of 1414 sequences were examined. The 148 collected specimens of birds from the province of Kabul, of which 38 were belonging to different non-asbestos orders, 110 belonged to the passeriformes, and other sequences belonging to the passeriformes in Kabul province were taken from the Gene Bank.
Discussion and Conclusion

The Taxonomic Situation of the Passeriformes

Passeriformes includes a mono-descendant species that seems to be a successful group of birds and rapidly separated in the late Tertiary period. According to the transitional classification based on the recognition of birds are divided into the subclasses (Tyranny) and Oscines (Passer), they were introduced with Acanthisittidae’s recent molecular studies as the third chord and the sister group for the two previous clusters, and its molecular traits (Johansson et al., 2001) [22].

On the basis of recent molecular studies a canthi’s titidae was introduced as the third substance and sister group Johansson, at al 2001) [22].

The Oscines group, with more than 4500 species, is the largest group with highest global distribution and probably originated from the Australian Region (Johansson, 2008) [22].
The division of Oscines into tow sisten taxon Sibley and Ahlquist 1990, [34] which was rejected in the recent years, and Corvida was introduced as a paraphilthic group (Barker et al., 2002; Ericson et al. 2002) [10].

Sibley and Ahlquist (1990). [34] defined the cluster for Passerida, including Passerioidea and Sylvioidae, and established Muscicapoidea as a regular group for two other clouds.

In addition to above contradictory phylogenetic hypothesis has been reported for phylogenetics, subs groups especially with passerida including with muscicapoidea-sylvioidae and passerida families (Ericson and johansson, 2003) [16].

Monodescendant (Sibley and Monroe, 1990) [35]. For example, the sequences of the RAG1 and RAG2H markers showed that Paramythiamontium, Toxorhamphus, Oedistoma and Melanocarhis, belonging to the Corvina nucleus in Sibley and Monroe (1990) [35] in the Passeria group (Barker et al., 2004) [11]. Also, molecular studies have shown that taxa that are not in the Passerida group may be part of the split. For example, Callicipaca, Chloropris, Irena, and some other species that were basically in the scavenger group are in the Passeria group (Barker et al., 2004) [11]. The Pseudopodoceshumilis species previously categorized in Corvini (crows) is associated with recent studies in the Passeria family (James et al., 2003) [21]. So far, many molecular studies have been carried out on passeriformes, but the largest number of taxon studied from passeriformes has been studied in research is 173 taxa of passeriformes (Beresford et al., 2005) [11]. Typically, most studies have been based on one or more nuclear genes as phylogenetic markers, and the use of several molecular markers has been proven, but so far few studies have used the combination of more than two molecular markers (Moore, 1999) [27].

Bayesian tree analysis of the COX1 mitochondrial gene

The bayesian tree was mapped using the amplified COX1 gene sequences from the Kabul province and the gene bank. The positioning of genera and species within families and superfamilies’ is similar to the results of DNA hybridization studies, conducted by Sibley and Ahlquist (1990) [34] for sparrows. Sparrows are a very large single-species group whose relationships are well known at least at higher taxonomic levels by DNA sequencing. However, new studies show that many families of sparrows in the traditional classification are not monophyletic. Understanding of the phylogeny of sparrows is possible when more extensive gene sequences are available. Based on DNA hybridization (Sibley and Ahlquist, 1990) [34] and nuclear gene sequences (Barker et al., 2002) [10], three major clades of sparrows have been shown to conventionally include Sylvioidae, Muscicapoidea and Passeroidea. The final bayesian tree of this research is also shown.

Passer genus with four species, Motacilla genus with one species, Carduus genus with one species, Emberiza genus with six species and Serinus genus with one species included in the family Passerioidea. Corvusgenus with one species and Laniusgenus with one species are also found in the Corvina clade. Two species of Luscinia with one species of Muscicapa and one species of Acridotheres belong to the family Muscicapidae. Two species of Hirundo, one species of Phylloscopus, one species of Riparia, one species of Sitta, and one species of Sylvia and one species of Eremophila are located in super-family Sylvioidae.

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