



Morphological and molecular identification and classification of Passeriform birds in Kabul, Afghanistan

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ABSTRACT

It is thought that the Passeriformes order of bird has monophyletic origin. However, new studies show that many passerine families are not monophyletic in traditional classifications. A more complete understanding of Passeriform phylogeny is possible only by conducting extensive molecular studies. Therefore, this molecular study was conducted to evaluate the Passeriformes birds of Kabul Province, Afghanistan. Samplings were collected from ten stations during three stages during the year 2019 and 2021 in Kabul province, including DehSabz, Bagrami, Sarubi, KhakJabar, Shekar Dara, Gol Dara, Paghman, Char Asiyab, Andrabi Road and Kafroshi. Totally 190 samples were taken using a mist net out of which only 110 samples were taken to the laboratory. After the morphological studies, it was identified by the identification key that all the species belong to the Passeriformes order belonging to 13 families, 23 genera and 35 species. After performing morphometry, the samples were transferred to the laboratory for molecular studies and DNA extraction and *COXI* gene sequencing to identify the species in the tree drawn by Bayesian method which shows the position of genera and species within families and superfamilies. In this study, genus *Passer* with four species, genus *Motacilla* with one species, genus *Carduelis* with one species, genus *Emberiza* with six species and genus *Serinus* with one species were included in the super family *Passeroidea*. The genus *Corvus* with one species and the genus *Lanius* with one species were included in the *Corvida* clade. Two species of the genus *Luscinia* with one species of the genus *Muscicapa* and one species of the genus *Acridotheres* were included in the superfamily *Muscicapoidea*. Two species of the genus *Hirundo*, one species of the genus *Phylloscopus*, one species of the genus *Riparia*, one species of the genus *Sitta*, one species of the genus *Sylvia* and one species of the genus *Eremophila* were included in the super family *Sylvioidea*.

Key words: *COXI* gene, Kabul, molecular classification, passeriformes

INTRODUCTION

Birds, with more than 10,000 species, constitute a diverse group of vertebrates in all aquatic and terrestrial ecosystems of the globe (Britannica ISLS, 2008). This category of vertebrates with their ability to fly, which makes them unique among other vertebrates, are scattered in various habitats of Afghanistan every year. Kabul province is one

of the most important provinces in the country in terms of protection of seasonal migratory birds and their habitats. The presence of the Hindu Kush and the Pamir Mountain range in the east of the province indicate the extra ordinary habitat diversity for birds. Kabul province has an area of about 3128 square kilometers, equivalent to half of the country's total area (Nuristani, 1971). Kabul province has a dry climate with average temperatures between -17°C

and 40°C rainfall 100 to 150 millimeters per year. Afghanistan assumed to be one of the most enriched sources of natural resources including wildlife. Birds in turn is a main portion of the resources and need to be scientifically identified for successful management and improvement (breeding) of biodiversity programs. In ancient times the common way for identification and classification was the morphology while in recent years molecular studies were entered to the classification field, in which organisms are classified accurately and precisely (Mohanta et al., 2012). Forests, fruit trees, and seasonal crops provide well living habitat for birds in terms of feeding and nesting (Khan et al., 2021). Afghanistan particularly Kabul province by having seasonal crops and trees that pave the way for feeding and nesting of different migratory and non-migratory birds. To manage birds successfully, the classification is essential to be science-based.

Though some of the foreign researchers morphologically studied and listed 483 bird species of Afghanistan, but data on molecular studies on

birds are unavailable. The goal of this research is to investigate and identify the birds of Kabul province based on morphology and DNA barcoding method by COX1 gene to place them in different clades. This research is done for the first time in the country in Kabul province.

MATERIALS AND METHODS

Place of study

Kabul province is located in the west of the Hindukush and the Pamir mountain range. This province has an area of about 3128 km², equivalent to half of the area of Kabul. Sampling was done from the Sanan in Kabul province and 7 cities, namely, DehSabz, Bagrami, Seroubi, KhakJabar, Shekardara, Gol Dara and Paghman. This sampling consisted of collecting feathers and muscles for molecular studies. Totally 190 samples were taken using a mist net out of which only 110 samples were taken to the laboratory for investigation. Sampling stations are shown in red on the map of Kabul province (Fig. 1).



Fig. 1. Map of Kabul province, the central part of Kabul, the areas from which the sampling was done are shown with red marks

Table 1. Details of sampling areas and the number of samples in Kabul province

Sampling location	Number of samples	Sampling date	Longitude	Latitude	Altitude
Die-sabz	5	2019	34°38'54.06"N	69°14'54.44"E	1748 m
Die-sabz	6	2020	34°38'54.06"N	69°14'54.44"E	1748 m
Die-sabz	35	2021	34°38'54.06"N	69°14'54.44"E	1748 m
Istalif	22	2021	34°83'44.72"N	69°14'40.61"E	1748 m
Istalif	15	2021	34°83'44.72"N	69°14'40.61"E	1749 m
Bagrami	4	2020	34°28'63.32"N	69°16'31.61"E	1791 m
Bagrami	1	2021	34°28'63.32"N	69°16'31.61"E	1791 m
serobi	5	2021	34°35'18.09"N	69°44'48.94"E	1001 m
serobi	5	2021	34°35'19.42"N	69°44'50.53"E	1001 m
serobi	5	2021	34°35'18.09"N	69°44'48.94"E	1001 m
Khaki-jabar	3	2019	34°20'00.02"N	69°26'00.09"E	
Char-asiab	5	2019	34°24'57.55"N	69°66'51.91"E	
Paghman	20	2021	34°35'05.24"N	69°58'41.62"E	2208 m
Guldara	9	2021	34°44'41.94"N	69°02'24.70"E	1991 m
Guldara	4	2021	34°44'41.94"N	69°02'24.70"E	1991 m
Shakar-dara	4	2019	34°41'51.09"N	69°04'18.87"E	1994 m

Study of morphometric traits

In nature and after releasing the bird from the net, many morphometric traits can be measured, including beak length, wing length, tarsus length, tail length, beak length, width, and height, as well as the wing formula, which includes the measurement of the longest feather length. It is primary and equivalent to giving it zero and the difference in the length of other primary feathers, especially P6 to P10 feathers. In the museum samples that the researcher has more time to measure, other traits can be added, including the length of fingers, knuckles, nail length, the maximum length of feather hair around the tip, etc. In this method, one of the valid identification keys is the identification key of Sanan sparrow species (Dementiev et al., 1996). And the bird atlases of the world and the neighbours whose birds are very similar to our birds.

Molecular method

DNA barcoding using DNA molecular markers has made it possible to identify and classify animals quickly and reliably, especially birds,

over the past years. In this method, the sequences of a small part of the mitochondrial genome (mt DNA) are used as an accurate and fast tool for identifying and classifying species. Based on this method, changes, order, number, and sequence of 650 standard gene nucleotides have been defined. Cytochrome oxidase-1 can indicate unique changes for each species. For this reason, this method is called barcode or DNA molecular identifier. COXI protein coding gene generally not only shows more differences at the species level compared to other mitochondrial ribosomal genes but is also more suitable for the diagnosis of very close species (Aliabadian et al., 2009, 2014).

Mitochondrial gene *COXI*

Now-a-days, cytochrome oxidase gene is used in molecular biosystematic studies. Among Kadine genes, this gene shows more differences between sequences than other genes. In recent studies, COX1 has been successful in distinguishing many closely related species in vertebrates and invertebrates. COX1 with 600 to 800 nucleotides in

the mitochondrial cytochrome oxidase gene region has been proposed to determine biodiversity in the world (Rubinoff, 2006).

According to Hebert et al. (2003), the reason for using this gene is the following:

- The presence of strong general primers for this gene, which can repair their 5-end, and these primers make PCR easier.
- It seems that this gene has more phylogenetic signs than other mitochondrial genes. The evolution of this gene is so big and fast that in addition to separating very close species, it also separates phylogeographic groups within a species.
- Baz substitution in the third nucleotide position is high in this gene (Hebert et al., 2003).

PCR Technique

Polymerase Chain Reaction (PCR) is a technique that is widely used in molecular biology. This technique takes its name from one of its key ingredients, DNA polymerase, which is used to make a large number of copies of a DNA strand. With the continuation of the PCR process, the number of initial copies of a DNA fragment is produced in a very large amount to the extent of several million copies, and the final product of PCR is generally called an amplification.

Bayesian analysis

It is a statistical method that uses an optimality criterion based on the state of attributes, with the difference that it does not search to find the best tree. In fact, in this method, based on information such as evolutionary model, branch length, and topology, and using the concept of the maximum likelihood, it draws a tree with the maximum likelihood. Bayesian analysis, like the maximum likelihood tree analysis, searches for a set of acceptable trees (Roderic and Edward, 1998). Bayesian analysis is used in molecular phylogeny, species phylogeny and species divergence time. This method uses a phylogeny tree and a model based on the expected value of the parameters and their maximum probability value, to arrive at a posterior

probability distribution for those parameters. The desired model is a tree with a specific topology, specific branch lengths, and a specific spatial model of DNA substitution and a specific distribution rate among nucleotide positions (Philippe et al., 2009).

The three methods mentioned above are discrete clustering methods. In general, the optimal tree method selects the tree or trees that have the least evolutionary changes. The most probable tree method selects the tree or trees that have the most probability among all the trees. Bayesian analysis performs the search by applying the maximum concept and targeting the probability distribution of phylogenetic trees (Hall, 2008).

Sampling method

An invisible net (Mist net) was used to catch specimens of this order in different habitats, and the specimens were captured by playing the sounds of the expected species in each habitat. After capturing the bird, it was separated from the net and before changing the position of its feathers, the necessary pictures were taken from its dorsal, ventral, and lateral surfaces. Then, some feather samples (so that they are not from the primary and secondary feathers of the bird) are separated and placed in a zipped bag. Care was taken that the shaft of the feather does not meet the hand. The number of the sample, the details of the sampling location, including the name of the location, the geographical location, and the gender of the bird were noted on the envelope, and the number and name of the bird were written in a notebook. Then, the tissue sample will be taken from the chest and muscle of the bird, transferred into vials containing alcohol, and the vial will be given a notebook number. Before releasing the bird, its biometrics were done, and the desired traits were measured by calipers with an accuracy of 0.01 recorded in the notebook. Morphometry includes body length, beak, tarsus, wings, and tail.

Sampling tools

Mist net, GPS, photographic camera, binoculars, containers containing alcohol, vials, envelopes, digital calipers, tools for dissection, plastic box, magic ruler.

Separation and storage of tissues for molecular studies: The tissues were kept in Merck alcohol. The size of the tissue should not be large because the water in the tissue dilutes the alcohol, it is better to change the alcohol in the vials containing the collected samples after a few hours. Different tissues such as muscle, liver, and kidney can be used for DNA extraction, but chest muscle tissue is usually used. The tissue pieces were separated from the tissue samples under completely sterile conditions and placed in another vial, and the sample number was noted on it. The parts should be small and large in number. Due to the large volume of DNA extraction and PCR steps, their data were omitted as and when required.

RESULTS AND DISCUSSION

The species identification based on morphological traits and creation of phylogenetic trees based on COX1 gene are presented. In the approach of morphological identification of the samples, the results were obtained based on keys and valid field guides, and in the molecular approach, the results were obtained using the gene sequences available in the NCBI and BOLD gene search engines. In this research, 1240 sequences from the gene bank and 86 sequences from 190 samples of birds captured from Kabul province were analyzed together in a molecular analysis. Finally, determining the position of Passeriformes in the related clades.

Identification of Passeriformes species in Kabul province

In terms of appearance, they are small or medium-sized birds, diverse in tip shape, with 10 to 11 primary feathers, with the first feather not developed, 9 secondary feathers and 10 tail feathers, with feet and 4 toes, with the first toe turned back. The forks are bent to different degrees. There is filling in all of them. Filling is done from the inside to the outside in the primary feathers and from the outside to the inside in the secondary feathers. In king feathers, the middle feathers are replaced earlier than the outer feathers. Their chicks are precocious.

Anatomically, they are birds with 14 cervical vertebrae, no petri-goid appendage, coccygeal gland, 5 true ribs, highly developed brain, no permon muscle, thumb flexor muscle not joined to finger flexor muscle (Dementiev et al., 1998). Passeriforms appeared 36 to 45 million years ago, at the same time as flowering plants and insects (Treplin et al., 2008).

The results showed that 190 specimens of Passerines and non-Passerine birds were captured during three stages during 2019 and 2020 years in 10 districts, 22 stages of field operations in Kabul province. Based on the morphological characteristics and using the valid identification keys of the existing samples, 110 samples belonged to the Passeriform order with 13 families, 23 genera and 35 species. The scientific names of the species by family are listed in Table 2. Also, the distribution map of these species and their characteristics are mentioned below.

Morphological characteristics of the distribution of captured species in Kabul province

***Lanius vittatus*:** This bird is 21 cm long and has a thick body, long tail, and big head. Forehead and eye band are black, tail and neck are gray, and in the male bird, the body is fawn red, the tail is white and gray, the undertail is white, and the breast and sides are pea white, which turns red on the sides. The chin and throat are white, the bill, legs and wings are black with a broad white wing band and the sides and tip of the tail are white. The young bird is generally pale with dark streaks on the sides, greyish white undertail feathers and dark brown wings with a small wing band. It is a species captured from Sarubi, Kabul.

***Lanius schach*:** This bird is 22 cm long and is clearly larger than the Oak-backed Eyestone, but its head is smaller. Also, the tail is longer, the edge of the feathers is pea-colored, the tail and the cover of the feathers on the tail are light orange (in the eye stone behind the oak, the tail is gray, and the cover feathers of the tail are white), the black eye line, which covers the top of the beak and forehead (but narrower). And the base of the feathers is marked with a smaller white spot (wing band), which is

sometimes absent (in an adult bird, the eye stone behind the oak is very distinct). White chin and throat, pea underbody, which is slightly fawn on the sides. The back of the neck is gray, the back is fawn red, and the tail is black. The young bird is paler, dark and grey brown on the back with dark brown spots and brown spots on the breast, underbody and tail. The sample was captured from Sarubi, Kabul.

***Oriolus oriolus*:** This bird is 24 cm long. He is shy and the same size as you, and except when migrating, his voice is heard more than seen. Feathers and wings of the female bird and the immature bird are green, the wings and tail are olive-brown, the tail is greenish-yellow, and the underbody is yellow white with darker streaks. In flight, the tail is relatively short and similar to woodpeckers, its flight is slightly wave-shaped, which changes its flight mode due to the slow tilting of the wings. It sits on the branches and usually at the top of the tree and hides among the leaves. The sample was captured from Sarubi, Kabul.

***Corvus monedula*:** This bird is 23 cm long and is one of the small black crows with gray back of the head and neck and both sides of the face and has colorless eyes and fast movements. The beak and legs are black, in flight, compared to other crows, it is smaller, its wings are faster, and its beak is shorter. It is often seen in a group with a black crow. The captured sample belongs to Herat, but it also exists in Kabul.

***Melanocorypha bimaculata*:** This bird is 16 cm long. And it is similar to the Chekauk, but slightly smaller, and in flight, it can be seen under the gray-brown wings, without the white border at the end of the secondary feathers. The tip of the tail is white, and the outer feathers are pea brown. On the ground, it is similar to Chekauk with the difference that the line under the throat is thinner, the underbody is white, the sides are pea brown, the eyebrow line is white, and the legs are fleshy red. Also, the head has dark and brown streaks more distinct than Chekauk's. The sample was captured from Kabul province.

***Alauda arvensis*:** This bird is 18 cm long and is characterized by a gray earthy color, pale brown

wing cover feathers, dark pea streaks on the body and a pea white underbody. Also, the chest with dark brown streaks on a pea background, dark with dark lines on a dark pea background, which at the end of the head, feathers are slightly prominent and turned into a round and short crest that is much smaller than the Chekauk crested crest. The relatively long tail, and the whiteness of its side feathers, can be clearly seen. The underside of the tail is black, and the wings are relatively long and elongated. It walks with a hunched posture and its flight is strong and slightly wavy. In flight, it has both in-situ wings and wing wings. The species is captured from Kabul.

***Calandrella brachydactyla*:** This bird is 14 cm long. Its size is small and pale. The trunk is linear, pale brown, under the trunk is a light pea with black spots on both sides of the throat (adult bird). The beak is hard and pale yellow, the cover feathers of the third largest wing, the white eyebrow line is relatively dark and dark with a black and narrow eye line, which covers the edge of the ear feathers. In an adult bird, the head and both sides of the chest are slightly mottled. It flies in waves and at a low altitude. The bird was captured from Paghman, Kabul.

***Eremophila alpestris*:** this bird is 16 cm long, its body is gray and dark brown, and the back of the neck is pinkish brown, and at the beginning of the neck, a narrow black stripe can be seen that leads to two black crown-like feathers on the sides of the head. The chin is white, the forehead and eyebrow line are white, which covers the border under the eyes and the black band on the chest, the underbody is white, and the legs are blue gray. The immature bird looks spotted and dark. The head markings in the adult bird fade slightly in winter. The seeded species is from Kabul province.

***Hirundo rustica*:** this bird is 16 cm long (including the two-branched tail) and due to the blue-black color of the body, the long two-branched tail (in which small white spots can be seen when the tail is wide), the throat and Oak forehead, dark breast band and pea underparts are easily recognized. The young bird lacks tail branches, but the breast and wing stripes are brown, and the forehead is

pale oak. His flight is strong and magnificent. It often comes close to humans and suddenly returns. It often hunts insects above the ground. The sample is made from Sorubi cable.

***Phylloscopus inornatus*:** This bird is 10 cm long and looks like Hume's leaf beetle (*Phylloscopus humei*) (even without distinguishing from each other). The color of the eyebrow is yellow (although it is often seen as grayish yellow), the body is gray green, the underbody is white, the eyebrow stripe is yellowish-white and continuous and sometimes wide (in red pea color), and a faint line is occasionally seen in the tail. and has two yellow-white wing stripes. The base of the beak is paler than Hume's leaf beetle, which has a dark entire beak. The species is captured from DehSabz, Kabul.

***Sitta tephronota*:** This bird is 14 cm long. Feathers and pillows are similar to small loincloths, but significantly larger. It also has a larger beak, a thicker neck, and a very long and clear black eye band that widens towards the back of the head. Its behavior is similar to that of a small chameleon, but it is often seen on trees or bushes. This species was taken from the straw shop in Kabul.

***Acridotheres tristis*:** This bird is 23 cm long and looks like a gray myna (*A. ginginonus*). But the feathers are reddish brown. The beak and the bare spot under the eye are bright yellow, and in flight, a distinct white spot at the base of the feathers (on both sides of the wing) is clearly visible. In the sitting position, white can be seen on the outer part of the underwing coverts and on the single tail feathers, except for the middle two feathers. The trunk is darker than the underbody and the tail is almost black. Usually seen in pairs or in small groups. His behavior is like a starling, but he is tame and fearless. The bird was captured from Kabul province.

***Luscinia megarhynchos*:** This bird is 18 cm long and its head is black, it is smaller than Mina. This bird is 16 cm long and is very similar to the Spotted Nightingale, but its body is lighter, more pink-brown, and its tail is red, and the white ring around its eyes is often seen more clearly. The underbody is clean and has no stains on the chest and sides. The subspecies that is found in Central

Asia and migrates to the East of the Middle East has larger cover feathers and the ends of the feathers on the edge of its wings are lighter and its lower body is also lighter. The eyebrow line is pale, and the rostrum is slightly pink, but mostly gray brown. The young bird is spotted, and the wing has pea spots on the chest and sides and pale spots on the middle cover of the wings. It is a species captured from Sarubi, Kabul.

***Luscinia svecica*:** This bird is 14 cm long and looks like a red breast, but it is a little thinner with longer legs and is often very shy. The eyebrow band is wide and clear, and the base of the tail is red in flight. When sitting, it holds the tail slightly high. In the breeding season, in the male bird, the chin and throat are blue, and the black and red oak lines are separated from the lower part of the breast below. In black and red oak, it is separated from the lower part of the chest. In autumn, the throat turns blue-white. Depending on the breed, sometimes the spot is white, red or absent. Immature birds of both sexes are seen with a pale throat. The young bird is spotted and is distinguished from the red-breasted young bird by its darker appearance and streaked streak and red tail. The captured samples are from Sarubi and Sabz, Kabul.

***Passer domesticus*:** This bird is 15 cm long and is well known. The male bird has gray plumage, oak brown sides of the head, pale gray cheeks, black chin, gray tail, oak brown wings and coverts, and a wide white stripe on the wings. The female bird and the young bird do not have contrasting colors on the head and throat and are seen with a pea-brown body and a pale gray underbody and a pale cream eyebrow stripe, and it is difficult to distinguish them from the female, black-breasted sparrow. The bird is social and reproduces in groups. The bird was captured from Kabul province.

***Passer hispaniolensis*:** This bird is 14.5 cm long and looks like a house sparrow (in some areas it is hybridized with it), but the male bird has a reddish-brown tail, a large black chin, and bright black streaks on the chest and sides. The body is streaked with black, which extends all the way around the underbody, and the cheeks and belly are white. The

female bird and the young bird cannot be easily separated from the female house sparrow. The lower body is whiter and gray streaks can be seen on its chest and sides. It is a social bird and is seen in groups mainly during migration and transit. The sample was captured from Kabul province.

***Passer montanus*:** This bird is 14 cm long and is smaller and prettier than the house sparrow, with a dark oak brown and two small black spots on its white cheeks, and the black on its chin is smaller. It also has two white wing stripes, and its tail is grey-brown. The sample was captured from DehSabz, Kabul province.

***Motacilla flava*:** This bird is 16.5 cm long. The color of the head in the male bird is different according to the breed (8 breeds have been seen in the Middle East region). The flava breed is seen with a dark blue-gray color and no obvious color change in the trunk, but in contrast with the dark gray ear feathers, and usually lacks an eyebrow line or white chin and throat. The female bird, in all breeds, is browner with less yellow underbody and reddish. During migration, it is difficult to distinguish races from each other. Race interbreeding among subspecies has created a variety of colors in this bird. The seeded sample is from Sarubi, Kabul.

***Anthus campestris*:** This bird is 16.5 cm long and is distinguished from smaller pipits by its relatively long tail, legs and beak, slightly striped trunk, and almost unspotted chest, with a simple sandy color. The young bird, in the first autumn, has a striped chest and no side stripes. Its wavy flight pattern is reminiscent of the yellow-bellied tern's tail. It walks quickly on the ground and makes sudden stops, so that, in that case, it keeps the body slightly upright. The sample captured is from GolDareh, Kabul.

***Anthus pratensis*:** This bird is 14.5 cm long. Its size is more delicate than the tree pipit and its tail is slightly longer. Its voice is the best feature of this tree pipit bird. In the first autumn, it is distinguished from the Red-throated Pipit by its unstriped bill (the red-throated Pipit has many stripes), the mantle with fewer stripes, the rump with a less pronounced appearance, and the eyebrow line that is not well defined. Chest with black streaks, pea-white underbody, white undertail and tail feathers, fleshy brown legs, and relatively long rear

toenails. Lesser than a pipit, it sits on a tree and its flight is wavy and irregular, and on the ground, it nervously wags its tail. The sample was captured from DehSabz, Kabul.

***Serinus pusillus*:** This bird is 12 cm long. It is small and pure, with black streaks, a smoky brown head and chest, and a bright orange spot on the forehead. In flight, the body is dark, but the underbody and underwings appear pale. The young bird has a clean orange face and black and white striped body and underparts. Outside of the breeding season, it is seen in small groups on the ground or in the grass of seeded weeds and is very lively. The sample was captured from Kabul shop.

***Carduelis carduelis*:** Appearance characteristics: This bird is 14 cm long and is seen with beautiful coloring and is recognized by the bright red and black and white color of the head, the yellow streak in the black wings and the white tail, which can be seen well in flight. The young bird (all breeds) has a pale gray proball and an unmarked head, and is identified only by its wing band, white bill, and voice. This bird is seen in small groups outside the breeding season. The sample was captured from Kabul city.

***Carpodacus erythrinus*:** Appearance differences: This bird is 14 cm long and is the same size as the red-breasted plover. The beak is relatively strong, the body is big, and the head is round. The immature bird is recognized by its red head, breast, and tail, which contrasts with the brown rump. The female bird and the young male bird, in the first summer, are paler and olive-brown in color. The upper body is slightly streaked, the lower body is much more streaked, dark in color with two white or pea stripes. The head is uniformly dark, and the eyes are also dark, and it is often singing at a certain point, its tail is heart-shaped at the end and its flight is wave-shaped. The sample was captured from Kabul city.

***Emberiza leucocephalos*:** This bird is 16.5 cm long and looks like a yellow lemon feather. In the male bird, a white spot with a black border can be seen on the bill and chin, but the head and throat are oak. Its color becomes lighter in winter. The female bird is gray-brown in color, dark, chest, flanks and front line, with a fawn tail similar to lemon feather. There

is a little white in the tail and a little oak color in the white of the throat, which is more in the female bird. Instead of yellow, the young bird has a white color on the belly and streaks on the primary wing feathers. The beak has two colors: the upper half of the tip is dark gray, and the lower half of the tip is pale gray. In the male bird of some intermediate breeds, the head, rump, and wing feathers are scattered with reddish-yellow, while, in others, the head is white. The species is captured from Kabul city.

***Emberiza cia*:** This bird is 16 cm long. In the male bird, the head is a mixture of black streaks on a smoky gray background with a streakless gray throat. The front is distinctly streaked with gray. The gray color of the bill and the white edges of the tail distinguish this bird from the African mountain tern and the house tern. The female bird is paler, with mixed colors and feathers. But basically, it looks like a male bird. The young bird is similar to the lemon-yellow young bird (both have brown oak bill). But the lemon yellowfin at any age has a large orange belly. The sample was captured from Kabul city.

***Emberiza stewarti*:** This bird is 16.5 cm long. The color of the head in the male bird varies according to the breed (8 breeds have been seen in the Middle East region). The flava breed is seen with a dark blue-gray color and no obvious color change in the trunk, but in contrast with the dark gray ear feathers, and usually lacks an eyebrow line or white chin and throat. The female bird, in all breeds, is browner with less yellow underbody and reddish. During migration, it is difficult to distinguish races from each other. Race interbreeding among subspecies has created a variety of colors in this bird. The species is captured from Shakar Dara in Kabul.

***Emberiza melanocephala*:** 16 cm; in the male bird, the ventral surface is uniform yellow, the head is black with a yellow collar, and the back is oak. There is no white color on its tail, its head is brown in autumn. The back surface of the female bird is olive brown with streaks. Among other yellow-breasted, yellow-breasted yellowtails, it is characterized by a ventral surface without streaks, and the cover of the feathers under its tail is yellow (refer to the red-headed yellowtail).

***Emberiza bruniceps*:** 16 cm; the male bird is easily recognized by its oak head and chest and bright yellow belly and tail. The female bird is very similar to the black-headed yellowtail female, but instead of the oak color, its back has a trace of green color, and the undertail feathers are white (not yellow). An immature bird is indistinguishable from an immature black-headed butterfly. It is a species captured from the sugar of the Kabul valley.

***Emberiza hortulana*:** 16 cm; It is distinguished by its pinkish pea-like ventral surface and yellow throat from the yolk of other moths. It has a pale olive-green head and chest, pale yellow throat, and olive stripe. Close up, the thin and yellow eye ring and pink beak can be seen, the dorsal surface is brown with black streaks. The female bird is lighter in color, its head is not very green, and dark and fine streaks can be seen on its chest. The immature bird is browner and streaked on the ventral surface, but its yellow eye ring and pink beak distinguish it. The sample was captured from Kabul city.

***Emberiza buchanani*:** This bird is 16 cm long and looks like a yellow-winged yellow-winged bird with a pink beak and a white eye ring. The head of the adult bird is smoky grey but lacks the gray chest band. The fawn's underparts are brown with a white throat and half-whisker-like stripe, the underbelly and undertail feathers are pea yellow or white. In autumn, the underbody of fawn is pea-colored with gray-brown streaks. The grayish-brown trunk contrasts with the oak color of the Bali strip. Veins in the upper body are either indistinct or absent. The bill is grey-brown, and the flight feathers are more gray-brown than black-brown. The edge of the wings is khaki. The young bird is seen with grayish brown tail and lower body and cover feathers under the tail. The sample was captured from Kabul.

Morphological results of captured species in Kabul province

From all the samples captured, a total of 6 morphological traits were measured with the help of a caliper and a ruler with an accuracy of 1%, 1%, respectively, length, beak length, beak height, beak width, tarsus length, wing length, and tail length. It is given in the table below (Table 2).

Table 2. Measurements taken on species captured from Kabul province and their number

No	Scientific name	Family	Number	en	tb	td	tt	hn	tn	tq
1	<i>Lanius vittatus</i>	Laniidae	1	5	90	115	33	9	14	215
2	<i>Lanius schach</i>	Laniidae	1	4	90	110	33	9	14	210
3	<i>Oriolus oriolus</i>	Oriolidae	1	4						
4	<i>Corvus monedula</i>	Corvidae	1	6						
5	<i>Alauda arvensis</i>	Alaudidae	1	6	100	75	27	7	17	180
6	<i>Eremophila alpestris</i>	Alaudidae	1	6	111	80	25	6	13	185
7	<i>Alauda gulgula</i>	Alaudidae	1	5	95	55	18	7	11	153
8	<i>Calandrella brachydactyla</i>	Alaudidae	3	6	120	70	30	7	18	175
9	<i>Hirundo rustica</i>	Phylloscopidae	1	5	116	115	13	2	6	170
10	<i>Phylloscopus inornatus</i>	Sylviidae	2	3	55	35	22	3	10	95
11	<i>Sylvia curruca</i>	Sittidae	12	3	65	55	23	3	8	130
12	<i>Sitta tephronota</i>	Sturnidae	1	6	82	50	30	5	30	155
13	<i>Acridotheres tristis</i>	Sturnidae	1	7	130	85	40	4	20	210
14	<i>Temeluchus pagodarum</i>	Muscicapidae	1	6	100	80	36	4	18	180
15	<i>Luscinia megarhynchos</i>	Muscicapidae	2	4	70	60	31	4	14	140
16	<i>Luscinia svecica</i>	Muscicapidae	2	4	75	60	30	4	14	140
17	<i>Muscicapa striata</i>	Phylloscopidae	1	4	67	50	22	4	12	120
18	<i>Passer domesticus</i>	Passeridae	12	5	77	60	22	7	10	145
19	<i>Passer montanus</i>	Passeridae	8	4	70	50	23	6	10	135
20	<i>Passer hispaniolensis</i>	Passeridae	3	5	75	60	22	7	10	145
21	<i>Motacilla flava</i>	Motacillidae	2	3	57	65	25	3	10	140
22	<i>Anthus hodgsoni</i>	Motacillidae	2	4	95	60	23	6	10	140
23	<i>Anthus pratensis</i>	Motacillidae	2	4	95	60	23	6	10	140
24	<i>Calandrella brachydactyla</i>	Alaudidae	1	5	90	55	21	5	9	135
25	<i>Calandrella</i>	Alaudidae	1	5	90	55	21	5	9	135
26	<i>Carduelis carduelis</i>	Fringillidae	2	8	80	55	22	7	12	135
27	<i>Pyrrhula pyrrhula</i>	Fringillidae	3	9	80	60	22	9	11	140
28	<i>Serinus pusillus</i>	Fringillidae	1	4	75	55	22	7	10	135
29	<i>Emberiza bruniceps</i>	Emberizidae	12	7	80	65	22	7	12	150
30	<i>Emberiza hortulana</i>	Emberizidae	2	5	80	75	21	5	9	140
31	<i>Emberiza buchanani</i>	Emberizidae	8	5	85	75	21	5	9	145
32	<i>Emberiza leucocephalos</i>	Emberizidae	1	5	85	80	22	6	10	160
33	<i>Emberiza cia</i>	Emberizidae	1	5	80	80	22	6	9	150
34	<i>Emberiza melanocephala</i>	Emberizidae	5	7	83	72	24	7	14	165
35	<i>Emberiza stewarti</i>	Emberizidae	9	4	85	70	18	4	8	145

(en: beak width, tb: wing length, td: tail length, tt: tarsus length, hn: beak height, tn: beak length and tq: height)

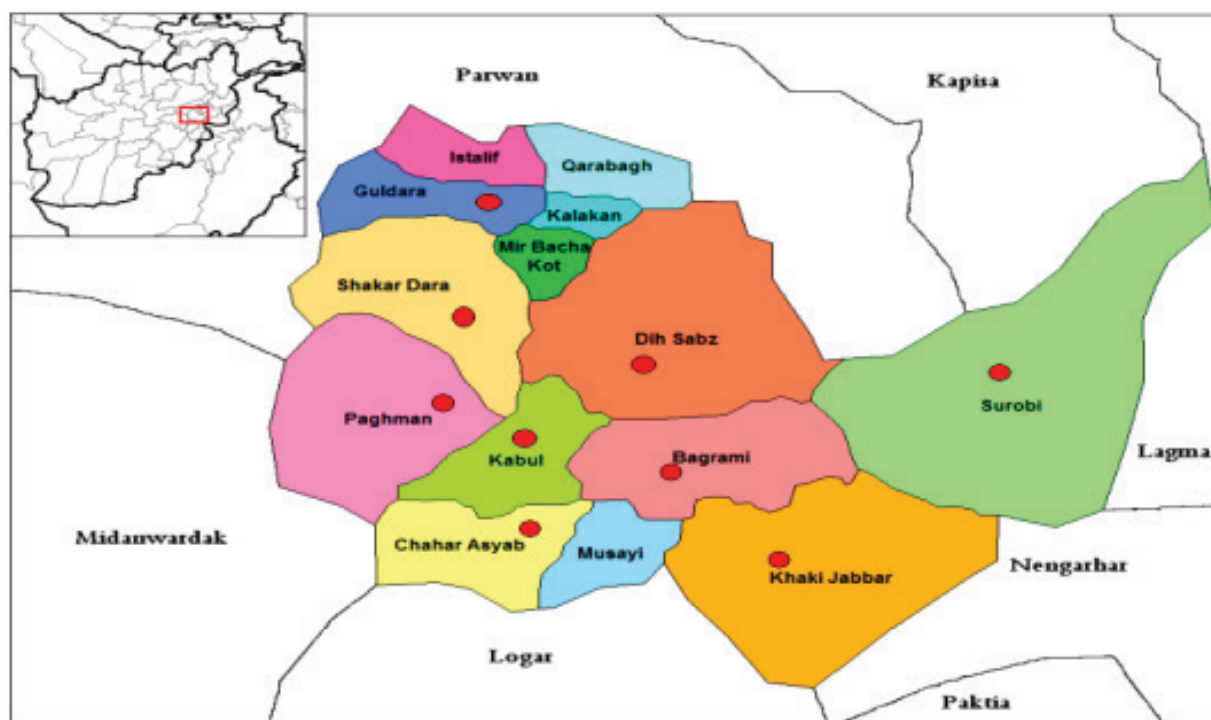


Fig. 2. The abundance and diversity of species captured in Kabul province

Morphological identification of Passeriformes species in Kabul province

Of the 540 species of birds that are mapped in "Birdlife" for Afghanistan, 280 species belong to 221 genera and 36 passeriform families of Afghanistan, including 150 species with 33 families belonging to the passeriform order of Kabul province. 110 sparrow hawks were captured during three stages in 2019 and 2020 in 10 districts and in 22 stages of field operations in Kabul province. They were selected for morphological and molecular study based on morphological traits and using valid identification keys from existing samples. These samples belong to the Passeriform order with 13 families, 23 genera and 35 species. During sampling, 30 species of them were morphometrically, the table of measured traits is given in (Table 2). Among the captured samples, the family of the yellow dragons had the most diversity with 7 species, and the family of the sparrows had the most abundance with 45 individuals, and other families showed less diversity and abundance than these two families (Fig. 2).

Taxonomic status of Passeriform order

Passeriforms include a tectonic group of bird species, which seems to be a successful group and rapidly diverged at the end of the Tertiary period. According to traditional classifications, based on the morphology of the voice box, they are divided into two clades: Suboscine (Tyranni) and Oscines (Passeri), with recent molecular studies, Acanthisittidae was introduced as the third clade and sister group to the previous two clades (Raikow, 1982) and its molecular features (Johansson et al., 2001) have been confirmed.

The Oscines group is the largest group with more than 4500 species and has a global distribution, and probably their basal lineages originated from the Australian region (Johansson, 2008). The division of Oscines into two sister taxa of Corvida and Passerida was done by Sibley and Ahlquist in 1990, in the following years this hypothesis was rejected and Corvida was introduced as a paraphyletic group (Barker et al., 2002; Ericson et al., 2002). Sibley and Ahlquist (1990) defined clades for Passerida that include Passerioidea and Sylvioidea and designated

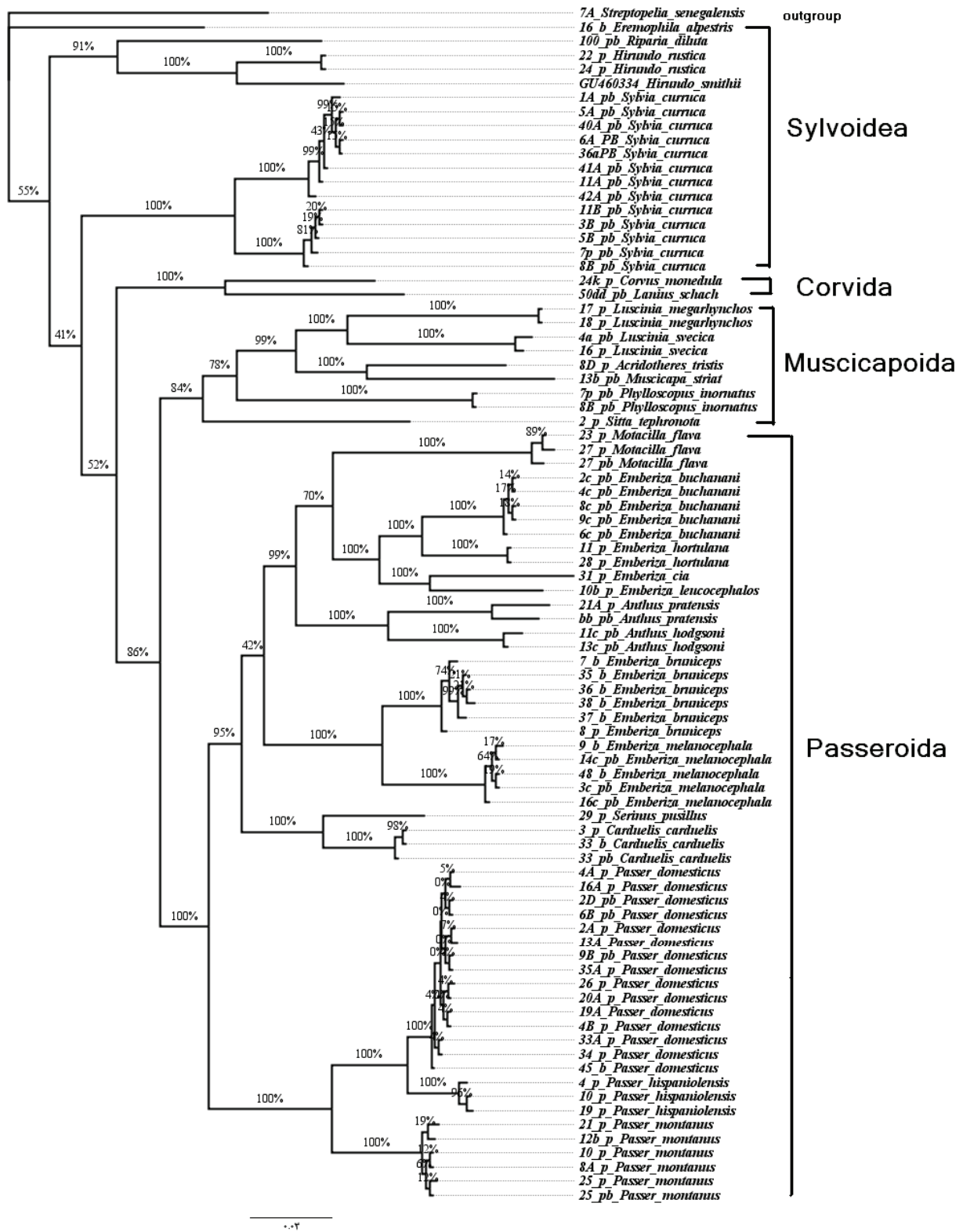


Fig. 3. Bayesian tree of captured passeriform species in Kabul province

Muscicapoidea as the basal group for the other two clades. In addition, conflicting phylogenetic hypotheses are seen for the phylogenetic relationships of lower groups, especially within the Passerida clade and its three superfamilies, including Muscicapoidea, Sylvioidea, and Passeridea (Ericson and Johansson, 2003). Although the hypothesis of Passerida being monotypic has been confirmed by many studies, all species and clades of Passerida have not been covered in the research (Sibley and Monroe, 1990).

For example, the sequences of RAGI and RAGII markers have shown that *Paramythia montium*, *Toxorhamphus*, *Oedistoma* and *Melanocharis*, which were classified in Sibley and Monroe (1990) in the Passerida group, belong to the Corvida core (Barker, 2004). Also, molecular studies have shown that taxa that are not included in the Passerida group may be part of this branch. For example, *Culicicapa*, *Chloropsis*, *Irena* and some other species that were traditionally in the flycatcher group have been placed in the Passerida group (Barker, 2004). The species *Pseudopodoces humilis*, which was previously classified in the Corvini (crows), has been placed in the Passerida family by recent studies. So far, many molecular studies have been carried out on the American sparrow, but the largest number of taxa studied from the American sparrow was in the research considering the number of 173 taxa of the American sparrow (Beresford et al., 2005). Usually, most of the studies have been based on one or more nuclear genes as phylogenetic markers, although the necessity of using several molecular markers has been proven, but so far, a few studies have used the combination of more than two molecular markers (Moore et al., 1999).

Bayesian tree analysis of the mitochondrial COX1 gene

Bayesian tree was drawn using the amplified sequences of COX1 gene from the captured species of Kabul province and gene bank. The placement of genera and species within families and superfamilies is similar to the results of DNA hybridization studies in Sibley and Ahlquist (1990) for sparrows. Passeriformes are a very large monophyletic group whose relationships, at least at higher taxonomic

levels, are well known by examining DNA sequences. However, new studies show that many passeriform families in traditional classification are not monophyletic. A more complete understanding of Passeriform phylogeny is possible when more extensive gene sequences are available. Based on DNA hybridization (Sibley and Ahlquist, 1990) and nuclear gene sequences (Barker et al., 2002), three major clades of sparrows have been shown, which conventionally include Sylvioidea, Muscicapoidea, and Passeroidea, which in the final bayesian tree of this research is also shown. The genus *Passer* with four species, the genus *Motacilla* with one species, the genus *Carduelis* with one species, the genus *Emberiza* with six species and the genus *Serinus* with one species are placed in the superfamily Passerioidea. *Corvus* genus with one species and *Lanius* genus with one species are included in Corvida clade. Two species of the genus *Luscinia* with one species of the genus *Muscicapa* and one species of the genus *Acridotheres* are placed in the superfamily Muscicapoidea. Two species of the genus *Hirundo*, one species of the genus *Phylloscopus*, one species of the genus *Riparia*, one species of the genus *Sitta*, one species of the genus *Sylvia* and one species of the genus *Eremophila* are included in the superfamily Sylvioidea (Fig. 3).

RECOMMENDATIONS

In the light of results of this study it is recommended to:

- Identify the birds of Afghanistan based on morphological and molecular methods.
- Register reports of the presence and absence of species and extensively investigate the status of reproduction, residence, wintering, or migration of birds.

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