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Phylogeny of Passeriformes (Aves) of Afghanistan with the Emphasis of Herat Province

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Abstract: ***Passeriformes are the largest group of birds all over the world, including about 60% of all living birds. Due to high degrees of similarityand convergent evolution between passerine's families, morphological studies yielded different results relative to DNA-DNA hybridization researches. Molecular studies supported the traditional classification and represented that Passerida is the largest group within Passeriformes, which constitute of three super families: Muscicapoidea, Sylvioidea and Passeroidea. Based on the researches, about 483 species are listed for Afghanistan, from which 140 species belong to Passeriformes captured from Kabul province. In the present survey 90 avian specimens were captured from thirteen localities in Heart province. COXI gene was extracted and sequenced from seventy individuals, which 69 individuals were belong to Passeriformes. In addition, 244 CoxI sequences of Afghanistan's passerines were retrieved from genbank and were added to our data. Our Bayesian analyses showed that from 28 studied families, monophyly of 17 ones were strongly supported, 2 ones represented with only one individual, one family was paraphyletic and the other eight families were polyphyletic groups. Besides, monophyly of all the three superfamilies of Passerida along with Corvoidea were not confirmed in our study.***

Keywords: Afghanistan, Heart province, Passeriformes, COXI, superfamilies.

1. Introduction

Aves constitute the important part of biodiversity which have evolved during million years. Arceopterix was recognized as the ancestor of aves, and then modern birds have been evolved (Grothand Barrowclough, 1990). Aves consisting of 10500 species in all aquatic and dry ecosystems and are one of the most diverse group of vertebrate on the earth. The birds which their ability of fly has distinguished them from other vertebrates, are distributed in diverse habitats of Afghanistan. Herat is one of the most important provinces in Afghanistan in terms of the protection of breeding and seasonal immigrant birds. Herat with 61315 km^2 is one of the largest province, after Kabul, in Afghanistan (Arianmal, 2012).

The largest and the most diverse group of the world's birds is Passeriformes which contain approximately 60% of all bird species distribute all of the continent except Antarctica (Sibley and Monroe, 1990). Passerines show the greatest species richness and range of ecological diversification among the other avian groups. Because of a lot of similarities and high degree of convergent evolution among passerine families, phylogenetic analyses of passerine based on the morphologic characters lead to some problems. So, these similarities and convergent evolution lead to the close relationship of passerine families in morphological analyses. However, DNA-DNA hybridization showed different phylogenetic relationship rather than morphologic characters. These results confirmed the traditional classification of oscines (suborder Passeri) and sub-oscine (suborder Tyranni) and proposed that oscines were contains two sister groups of Passerida and Corvida. Passerida is the biggest group within the passerines which divided to three super families; Muscicapoidea, Sylvioidea and Passeroidea (Wu et al., 2015).

Based on the available data, 483 bird species were listed for Afghanistan (Avibase, 2012), from which 140 species are belong to Kabul's passerine (Arianmal, 2012). Up to now no molecular studies have been performed on passerine of Herat province. Therefore, due to the frequency and diversification of passerine species in Afghanistan, Herat province is an appropriate place for molecular studies on passerine. So, the aim of this survey is the investigating of passerine in Herat province using molecular analyses on mitochondrial protein coding gene Cox1 and test the monophyly of passerine families and super families and phylogenetic relationship among super families.

2. Methods and materials

In order to investigate the passerines of Herat which is one of the largest province in the northwestern Afghanistan, some sampling were done in between April to September in 2017 to 2018 from thirteen stations of Karukh, Guzara, Injil, Gozare (Kurt), Pashtun Zarghun, Ghoryan, KushkiKuhna, Rozanak, AbMazar, Herat city, Adraskan, Gulran and Pole Rangine (Fig. 1 and Table 1), which ninety specimens were collected. Capturing of samples were performed using miss net.

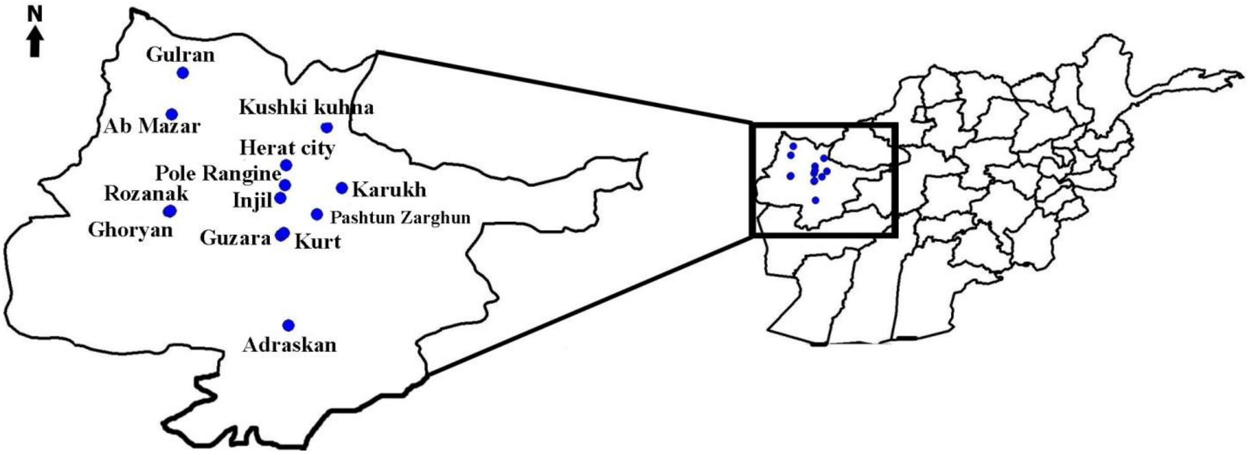

Figure 1: Geographical positions of sampling stations.

Table 1: Sampling localities of Passeriformes in Herat province of Afghanistan. In each location the number of collected samples and geographic coordination were shown

	Sampling locality	Geographic coordination		Number of samples
1	Pashtun Zarghun	34° 19´ 40 ″N	62° 26´ 51″E	15
2	Karukh	34° 29´ 26 ″N	62° 36´ 11″E	6
3	KushkiKuhna	34° 52´ 12 ″N	62° 30´ 23″E	10
4	Guzara	34° 11´ 54 ″N	62° 13´ 17″E	21
5	Kurt	34° 12´ 37 ″N	62° 14´ 18″E	1
6	Injil	34° 25´ 47 ″N	62° 12´ 57″E	3
7	Ghoryan	34° 20´ 32 ″N	61° 30´ 59″E	3
8	Rozanak	34° 20´ 55 ″N	61° 31´ 34″E	8
9	Gulran	35° 12´ 38 ″N	61° 36´ 15″E	1
10	AbMazar	34° 57´ 05 ″N	61° 31´ 59″E	2
11	Adraskan	33° 38´ 10 ″N	62° 15´ 58″E	12
12	PoleRangine	34° 30´ 40 ″N	62° 14´ 20″E	7
13	Herat city	34° 37´ 59 ″N	62° 15´ 15″E	1
				Total: 90

Some parts of muscle and breast tissues were separated and preserved in 96% ethanol. Seventy specimens were selected for molecular studies from which sixty nine individuals belong to passerine and one of them was a non-passerine. Genomic DNA was extracted using salt method (Bruford et al., 1992). Cox1 (Cytochrome Oxidase C, subunit I) gene was amplified using six primers (Table 2).

Table 2: The name and sequences of primers were used in this study.

Name of primer	Sequence of primer
PasserF1	5´-CCA ACC ACA AAG ACA TCG GAA CG-3´
PasserR1	5´-GTA AAC TTC TGG GTG ACC AAA GAA TG-3´
AWCF1	5´-CGCYTWAACAYTCYGCCATCTTACG-3´
AWCR6	5´-ATTCCTATGTAGCCGAATGGTTCTTT-3´
AF	5´-AAC CAA CCA CAA AGA CAT TGG-3´
AR	5´-CCA TGT AGC CGA ATG GTT CT-3´

PCR program for PasserF1 and PasserR1 was carried out in Primus 96 thermal cycles with an initial denaturation step at 95˚C for 3 min, followed by 40 cycles of 1 min at 94˚C, 40s at 58˚C and 90s at 72˚C, with a final extension time of 5min at 72˚C (Lohman et al., 2009). Amplification with AWCF1 and AECR6 contained a preliminary denaturation step of 94˚C for 2 min. Then followed by 35 cycles of 30s at 94˚C, 30s at 57.5˚C and 30s at 72˚C, and final extension of 4 min at 72˚C (Patel et al., 2010). Amplification conditions for AF and AR were as follows: an initial denaturation step of 94˚C for 2 min. Then followed by 40 cycles of 20s at 94˚C, 45s at 51˚C and 30s at 72˚C and final extension of 3 min at 72˚C (Park et al., 2011). One strand of PCR products were sequenced in Macrogen Company in South Korean and Microsynth in Sweden.

244 CoxI sequences of Passeriformes of Afghanistan were retrieved from genbank and were added to our data. Suences were edited by BioEdit 7.0.5 (Hall, 1999) and were aligned using CLUSTAL W algorithms (Thompson et al., 1994). Stop codons were omitted using Mega7 (Kumar et al., 2016). Bayesian inference (BI) was performed with MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003) using Markov Chain Monte Carlo methods. Four chains were run simultaneously for 10 million generations. The first 10000 trees as a conservative "burn-in" were discard. Mean genetic distances were calculated within and between species using Kimura-2-parameter in Mega7.

3. Results

Totally after editing and aligning the sequences, 795pbs of COXI gene were analysed.Sixty nine sequences belong to individuals of Herat province which were sampled in this study and belong to passerines and one to non-passerin, *Meropsapiaster*(Table 1) that was considered as outgroup. Bayesian tree was drown and depicted in Fig. 2.

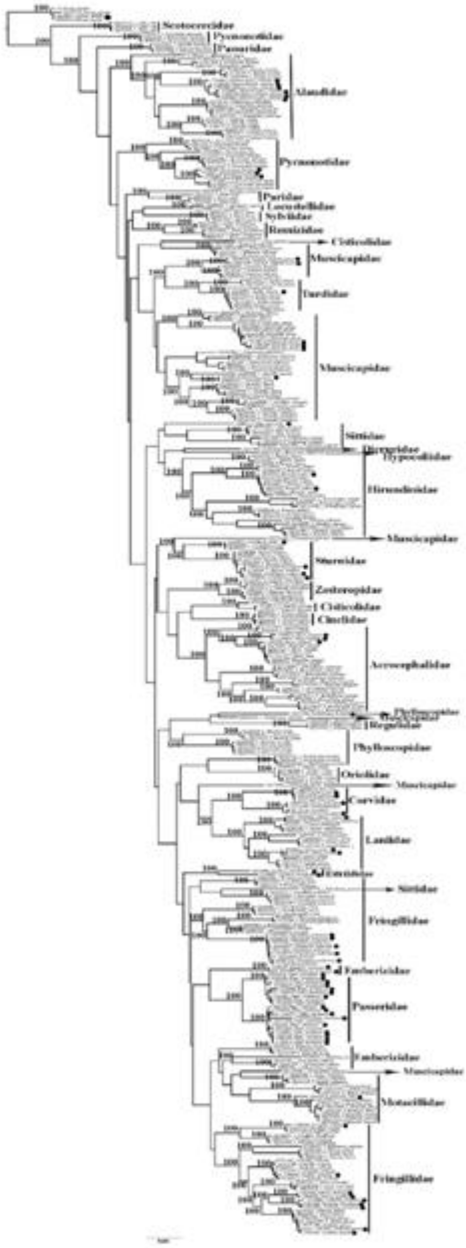

Figure 2: Bayesian tree resulted from CoxI gene data of passerine individuals from Afghanistan's passerines. The numbers showed posterior probabilities. (Samples were captured in this study were indicated by black circles).

Our samples with genebank sequences altogether comprised 28 families of Passeriformes of Afghanistan which were analysed in Bayesian method. In the Bayesian tree, 19 families showed monophyly with the highest support of posterior probabilities (100%), except Dicruridae and Hypocoliidae which had only one specimen.However eight families of Pycnonotidae, Paridae, Cisticolidae, Muscicapidae, Sittidae, Phylloscopidae, Fringillidae and Emberizidae were polyphyletic groups, and one family, Motacillidae was paraphyletic.

Pairwise genetic divergences were calculated between specimens and were represented by a diagram in Fig. 3.

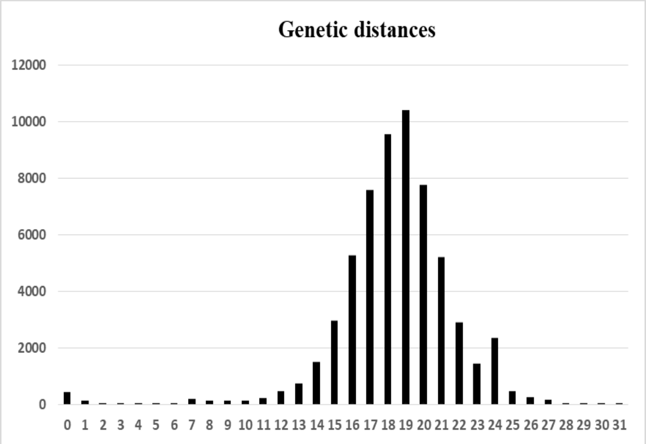

Figure 3: Diagram of pairwise genetic distances. Horizontal axes showed genetic distances and vertical axes represented the frequencies of each distance

Based on this analyses, intraspecific genetic divergences ranged from 0% to 8%, interspecific genetic divergences from 0% to 12% and intergeneric divergences ranged from 6% to 31%.

4. Discussion

Passeriformes is the largest order within the class Aves which diverged late of Tertiary very fast and successfully (Feducia, 1995). Morphological and molecular data represented their monophyly very well. In traditional classifications based on the vocal box, passerines divided intotwo clades: Suboscines (Tyranni) and Oscines (Passeri). Sibley and Ahlquist (1985) divided Oscines to two sister taxa Corvida and Passerida, but this classification have been rejected and Corvida have been recognized as a polyphyletic group. Sibley and Ahlquist (1990) recognized three clades within Passerida: Passerioidea, Sylvioidea and Muscicapoidea, with Muscicapoidea basal relative to other two clades. However,several taxonomic hypotheseshave been stated for phylogenetic relationships of lower groups in these superfamilies (Johnsson et al., 2008). DNA-DNA hybridization (Sibley and Ahlquist, 1985), nuclear genes sequences (Barker et al., 2002) and ribosomal RNA also confirmed the existence of three main clades in Passerida (Wu et al., 2015). Although monophyly of Passerida is confirmed in several studies, but all species and clades in this taxon are not monophyletic taxa (Sibley and Monroe, 1990).

Until now there have not been any comprehensive study on Afghanistan's birds, except a few reports by foreign scientists.For example in "Birds of Minor Asia" 157 breeding species belong to 23 families of Passeriformes (Aye et al., 2012) and in other study 254 breeding species belong to 34 families of this order have been recorded from Afghanistan (Avibase, 2012).

Phylogenetic relationships within Muscicapoidae

Four families of Muscicapoidea:Sturnidae, Turdidae, Cinclidae and Muscicapidae were included in our Bayesian analyses as the Afghanistan birds' fauna. From which the first three of them are monophyletic but Muscicapidae show polyphyly. Muscicapidae is a diverse taxon contain 48 genus with 275 species of birds (Clement, 2007). However because of the lack of precise phylogenetic data, their monophyly is unknown yet (Sangster et al., 2010a). Analyzing of combined data of mitochondrial and nuclear genes, proved that Muscicapoidea is polyphyletic and some its genus are not belong to this superfamily. In this survey, Turdidae and Sturnidae were sister group to Muscicapidae (Voelker and Spellman, 2004). Other studies based on molecular data showed that Muscicapoidaeis not monophyletic and their species are placed in two subfamilies of Muscicapinae and Saxicolinae (Sangster et al., 2010b). In our study, Muscicapinae with one genus *Muscicapa* is monophyletic, but Saxicolinaeformed polyphyletic group.

Phylogenetic relationships of Sylvioidea

Phylogenetic position of Sylvioidea is unclear and no study suggested monophyly of Sylvioidea (Alström et al., 2006; Sibley and Ahlquist, 1985). In bayesian analyses on Passerifomes from Kabolprovince of Afghanistan, Arianmal (2012) did not found monophyly for Sylvioidea. While, comprehensive study in 2012 with the most numerous taxa of Sylvioidea, strongly supported the monophyly of this superfamily. Based on that study, Sylvioidea comprising of 23 families, 220 genus and 1200 species (Fregin et al., 2012). Our results disagree with Fregin et al. (2012) in supporting the monophyly of Sylvioidea. Eleven families of Sylvioidea are included in the present study, from which two families Pycnonotidae and Phylloscopidae were polyphyletic, while the monophyly of others were supported by high posterior probabilities.

Phylogenetic relationships in Passeroidea

Passeroidea are the largest superfamily within Passerida which occupy all available habitats of the world and inhabit all continents except mainland Antarctica (Sibley and Ahlquist, 1990). Generally, monophyly of Passeroidea has been supported by several studies, but all the species and clades included in the Passerida have not been confirmed to be part of this clade. For instance, Sibley and Ahlquist (1985) suggested that Alaudidae placed in the Passeroidea, otherwise, recent studies changed its phylogenetic position and placed this family in Sylvioidea (Johansson et al., 2008).

In our phylogenetic tree, there were five families of Passeoidea which occupy habitats of Afghanistan: Passeridae, Fringillidae, Emberizidae, Estrildidae and Motacillidae. Monphyly of Passeridae and Estrildidaewerestrongly supported (100%) in our results, Fringillidae and Emberizidae showed polyphyletic statue and Motacillidaewas paraphyletic.

Phylogenetic relationships in Corvoidea

Recent studies based on the nuclear genes, were disagree with monophyly hypothesis of Corvoidea and suggested it as a polyphyletic group (Barker et al., 2002). Our results also supported these results and represented that the four families included in our analyses, Corvidae, Laniidae, Oriolidae and Dicruridaedid not constitute a monophyletic group. Our Bayesian tree sowed the sister group relationship between Corvidae and Laniidae with high support. Oriolidae was a monophyletic taxon, and there was only single specimen of Dicruridae.

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