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Short Communication

Molecular Identification of Pakistani Lathy Rock Pigeon using Cytochrome B Gene

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SF, MUL and ARA designed the study, analysed the data and wrote the manuscript. MT, SS and MW edited and revised the manuscript. IR collected the samples.

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Domestic lathy pigeon, Mitochondrial DNA, Cytochrome B, SNPs, Phylogenetic analysis.

ABSTRACT

Single nucleotide polymorphism (SNPs) analysis is a powerful tool for taxonomic and phylogenetic analysis. These are bi-allelic molecular markers, easy to interpret and uniformly distributed within genomes. In this study cytochrome b (*Cyt*b) gene based SNPs analysis was employed to genetically characterize the Pakistani domestic breed Lathy pigeon. *Cyt*b haplotype for local domestic breed was developed. Homology analysis of *Cyt*b gene revealed *Columbia livia* as closest homologue of Pakistani Lathy pigeon with five novel SNPs. Phylogenetic analysis of domestic lathy pigeon indicated the monophyletic relation with *C. livia*. We are reporting for the first time, the novel SNPs in the *Cyt*b gene of local domestic pigeon breed that might act as panel of molecular marker for the identification of Pakistani lathy pigeon.

Pigeon have played significant role throughout the human history, interacted with human for a variety of purposes and are being used as pet for racing competitions as a source of amusement and sports in many countries of the world. Some varieties of pigeon used in racing competitions are very costly and are of high economic value, due to which stealing, smuggling and mugging issues are common for these pigeons. Apart from these issues, the specie and genetic lineage of these pigeons is also questioned very often. So, it is very important to develop panel of genetic markers to identify unambiguity of the breeds (Lee *et al*., 2008). Pakistan is bestowed by a diversified avian fauna (Awan *et al*., 2013). Among them pigeons are distinctive birds which have been bred for centuries. Lathy Rock is a unique Pakistani domestic pigeon; colloquially is Salara in Punjab. The Lathy Rock is semi-solid in color having light and dense gray feather pattern throughout the body. They have orange colored iris with golden paler inner ring (http://faisalabad.olx.com.pk/). Traditionally, they have been identifying on the basis of morphological and anatomical characteristics (Danish *et al*., 2008). No molecular data is available to authentically identify this pigeon breed.

DNA based molecular markers provide new scaffold for critique breed identification (Tautz *et al*., 2003). Among these molecular markers, mitochondrial *Cyt*b gene has proven to be an efficient tool for species identification and characterization (Firyal *et al*., 2013; Saif *et al*., 2012). *Cyt*b gene has been extensively employed to resolve relationships between closely related taxa and to build higher level phylogenies of vertebrates (Johns and Avise, 1998). Phylogenetic analysis is an imperative molecular tool which can underpins the taxonomic affinities, molecular diversities and lineage assignment of avian species (Allan and Max, 2010). As no documentation is available for Pakistani pigeon breeds herein we describe through DNA sequencing, the gene specific novel polymorphisms in *Cyt*b gene of the Pakistani domestic pigeon; Lathy Rock and its molecular classification within the genetic spectrum of other Columbiformes.

Materials and methods

A total of 23 unrelated domestic lathy pigeons were selected on the basis of yellowish iris color and dense gray feather pattern throughout the body, from 3 cities (Lahore, Bahawalpur and Sialkot) of Punjab province of Pakistan. The samples were named as PKDRP1 to PKDRP23. Blood samples (200 µl) were collected from each bird and utilized for the isolation of genomic DNA (Sambrook and Russel, 2001). Primers were designed using the *Cyt*b gene sequence of *Columbia livia* (Accession No. GQ240309) as reference sequence. Full length *Cyt*b gene was amplified with the help of specific primers. Amplicons were purified

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and subjected for sequencing using an ABI Prism 3130 genetic analyzer (Applied Biosystems, Foster City, USA). The obtained DNA sequences were analyzed using various Bioinformatic tools. ClustalW (http://www.ebi.ac.uk) was used to perform the multiple sequence alignment. On the basis of pairwise alignments, phylogenetic tree was constructed with the help of NCBI distance tree matrix and Blast Tree View; in which the *Cyt*b gene sequences of the Lathy Rock pigeon was compared with all the available *Cyt*b gene sequences of Columbiformes (pigeons and doves). The nucleotide sequences of *Cyt*b gene of Pakistani Lathy Rock pigeons were submitted to GenBank using Sequin Submission Portal.

Results and discussion

From all the selected birds, PCR resulted in amplification of 1.143kb *Cyt*b gene. The *Cyt*b gene sequences of 23 domestic Lathy Rock pigeons were submitted to GenBank under the Accession No. KC811442 to KC811463. The sequence similarity analysis indicated *Columba livia* as closest homologue of domestic Lathy Rock pigeons with 99.61% homology. Table I demonstrated the percentage homology of domestic Lathy Rock pigeons with the various members of Columbiformes. Comparative analysis of *Cyt*b gene from domestic Lathy Rock pigeons and *C. livia* revealed the presence of 5 SNPs in the *Cyt*b gene (T174A, C232T, G261A, G703A and G816A). Phylogenetic tree constructed based on *Cyt*b gene polymorphisms of Lathy Rock with all available *Cyt*b gene sequences of the Columbiformes indicated that domestic Lathy pigeon is monophyletic with respect to *C. livia* and *C. rupestris* (Fig. 1).

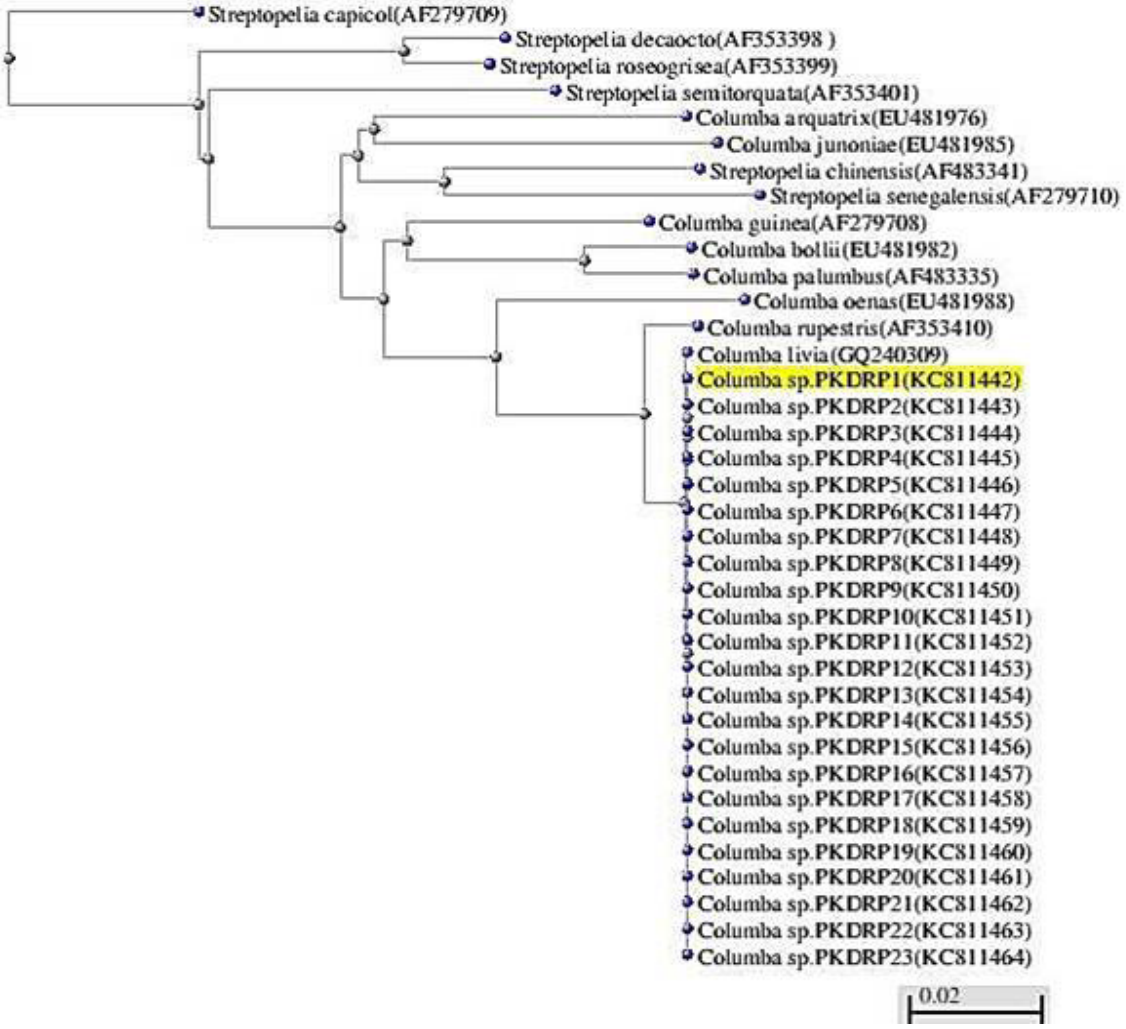

Fig. 1. Phylogenetic tree from Pakistani Lathy Rock based on comparison of *Cyt*b sequence of Pakistani lathy rock with the same gene from previously reported pigeon species available in the NCBI data base.

Table I.- Percentage homology of Pakistani domestic Lathy Rock pigeon with other members of Columbiformes.

Specie name	Accession No.	Percentage homology
C. livia	GU906131	99.61
C. rupertris	AF353410	98.5
C. oenas	EU481988	93.7
C. guinea	AF279708	92.0
C. bollii	EU481982	91.1
C. palumbus	AF483335	90.7
C. arquatrix	EU481976	90.5
C. junoniae	EU481985	90.2
Streptopelia chinensis	AF483341	90.2
S. senegalensis	AF279710	88.8
S. roseogrisea	AF353399	88.7
S. capicola	AF279709	87.0
S. semitorquata	AF353401	88.5
S. decaocto	AF353398	88.5

Present study describes the molecular characterization and phylogeny of Pakistani domestic Lathy Rock pigeons using *Cyt*b gene polymorphism. Mitochondrial *Cyt*b gene is extensively used to underpin the taxonomic classification and systematic resolution of an individual (Heidrich and Wink, 1998; Saif *et al*., 2012; Tautz *et al*., 2003; Firyal *et al*., 2013) and has been considered one of the most useful genes for evolutionary studies (Esposti *et al*., 1993). It has been extensively employed to address the diversity ambiguities as it contains both conserved and rapidly evolving codon positions (Irwin *et al*., 1991).

Comparative analysis of *Cyt*b gene from domestic Lathy Rock pigeons and *C. livia* divulged the presence of 5 SNPs in the *Cyt*b gene. These 5 SNPs in the *Cyt*b gene would prove to be novel for identification of Pakistani Lathy Rock pigeon breed.

Further, *Cyt*b gene polymorphisms based evolutionary analysis of lathy Rock explored the continuum of shallower to deeper genetic divergences. This unique continuum evolution defines the biological relationships of individuals. Phylogenetic tree constructed based on *Cyt*b gene polymorphisms of lathy Rock with all available *Cyt*b gene sequences of the Columbiformes indicated that domestic lathy pigeon is monophyletic with respect to *C. livia* and *C. rupestris* (Fig. 1). In addition to this, analyses of present study significantly revealed that rock pigeon (*C. livia*) is the ancestor of Pakistani domestic pigeons (Gonzalez *et al*., 2009; Levi, 1996; Stringham *et al*., 2012). Rock Pigeon and Pakistani domestic pigeons diverged from Hill pigeon (*C. rupestris*): likely to be allopatric. Branch lengths separating them are very short and believe to be a recent radiation. No empirical data is available about this radiation because little work has been undertaken to address this issue. These findings are unswerving even with the whole genomic studies (Shapiro *et al*., 2013). The present study provides the basis for further genetic investigations of avian species. This is the first report of novel SNP identification of *Cyt*b gene of the Pakistani Lathy Rock pigeon and its phylogeny.

Statement of conflict of interest

The authors have no conflict of interest and are agreed to submit this piece of information to Pakistan Journal of Zoology for publication.

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