



SHORT COMMUNICATION

Molecular Classification of Pakistani Domestic Pigeon using *Cytochrome b* Gene

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ABSTRACT

Pakistan has distinct domestic pigeon breeds which have been bred for centuries. This study aimed to identify novel Single Nucleotide Polymorphisms (SNPs) in Cytochrome b (*Cytb*) gene of a unique Pakistani domestic pigeon named as *Sherazi* for its molecular classification. The full-length *Cytb* gene of the Pakistani domestic pigeon ($n=25$) was sequenced. Five novel SNPs sites were identified in the *Cytb* gene of the Pakistani domestic pigeon after comparing with those of Rock Pigeon (*Columbia livia*). The phylogenetic analysis, using the *Cytb* gene sequences of Pakistani domestic pigeon and all the available *Columbiformes*, revealed that *C. livia* is the ancestor of the Pakistani domestic pigeon *albeit* the Pakistani domestic pigeon differs phenotypically from it. The novel SNPs in *Cytb* gene of the *Sherazi* pigeon can serve as a genetic marker for identification, phylogenetic analysis and evolutionary characterization of pigeon species. This is the first report of SNP identification of *Cytb* gene of the Pakistani domestic pigeon and its molecular classification.

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INTRODUCTION

The pigeons are the oldest domesticates (Driscoll *et al.*, 2009). Most of the pigeon breeds are reared exclusively because of their physical characteristic, soaring capabilities and for their racing-sports. Pakistan has distinctive domestic pigeon breeds which have been bred for centuries. *Sherazi* is a unique Pakistani domestic pigeon which is also named as *Lahori* in Pakistan and its relatives are known as *Shirazi* in Iran, *Saksarli* in Turkey and *Shaik* in Lebanon (<http://www.avianweb.com/shakhsharlitumblers.html>; 02-02-2013). The markings on the *Sherazi* pigeon make it a unique breed. The *Sherazi* is semi-solid in color from the bottom of the chest going up and around the neck in a smooth pattern. There are clearly identifiable color-patches on cheeks and skull. The color of the patches is same as the body color. All the patches are desired characteristics of the breed. No white feathers are to be present in the vent area. The *Sherazi* pigeon is considered as a good flyer (<http://wwingsaviary.lbbhost.com/PigeonPages/Shakhsharli.html>; 02-02-2013). Taxonomic characterization of pigeons always remains a challenge for taxonomists, which raises the question about the ancestry of domestic pigeon breeds and their lineage diversity. Phylogenetics is an imperative molecular tool

which can explore the range of genetic divergences between taxa, delimitation of bird species and their molecular evolution (Gibb and Penny, 2010; Kan *et al.*, 2010).

Among mitochondrial genes, *Cytb* gene markers have been proved as an efficient tool with high power of discrimination for species identification and characterization (Saif *et al.*, 2012). As no documentation is available for Pakistani pigeon breeds herein we describe through DNA sequencing, the gene specific novel polymorphisms in *Cytb* gene of the Pakistani domestic pigeon; *Sherazi* and its molecular classification within the genetic spectrum of other *Columbiformes*.

MATERIALS AND METHODS

To explore the polymorphisms in *Cytb* gene of Pakistani domestic pigeon; *Sherazi*, blood samples from twenty five unrelated pigeons with typical phenotypic features were selected from different regions of Punjab, Province of Pakistan. The samples were named as ARA-2012-PKPSH1 to ARA-2012-PKPSH25. The DNA from the samples was extracted by the standard organic method. Primers were designed using *C. livia* mitochondrial sequence available (NCBI GenBank; Accession no. GQ240309). The specific primers were

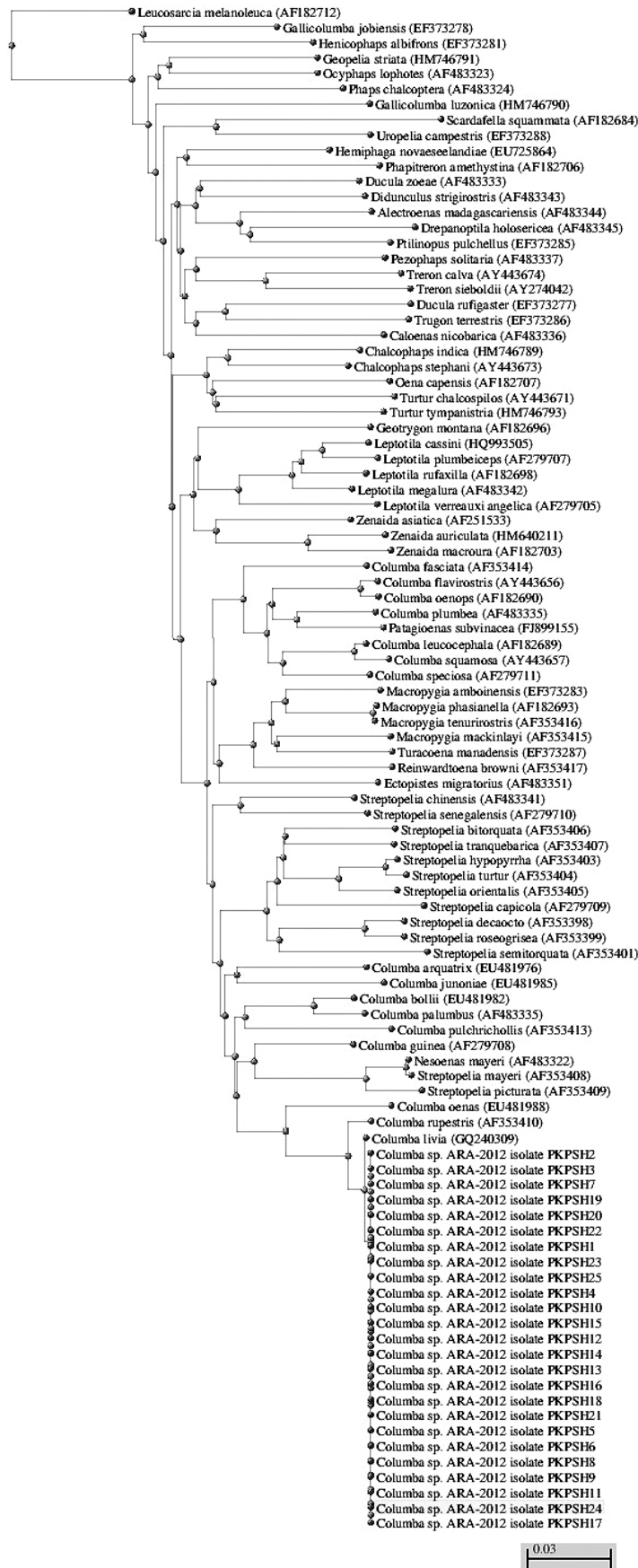


Fig. 1: The phylogeny based on the *Cytochrome b* gene sequences of *Columbiformes* indicating the phylogenetic and molecular classification of the Pakistani domestic pigeon.

Table 1: Single Nucleotide Polymorphism in *Cytochrome b* gene of the Pakistani domestic pigeon

No.	Allele	Base position	Rock Pigeon	Pakistani Domestic Pigeon
1	T/A	113	T	A
2	C/T	171	C	T
3	G/A	200	G	A
4	C/T	521	C	T
5	G/A	642	G	A

used for amplification of full-length *Cytb* gene through Polymerase Chain Reaction (PCR). Purified PCR products were sequenced with both forward and reverse primers using BigDye terminator cycle sequencing kit (Applied Biosystems, USA) on ABI 3100 Genetic Analyzer. Sequence data was edited manually using Chromas Ver. 1.45, (http://www.tech_nelysium.com.au/chromas.html). Nucleotide sequences of *Cytb* genes of the Pakistani domestic pigeon were submitted to NCBI GenBank. Multiple sequence alignments were performed with NCBI BLAST freeware (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). A phylogenetic tree was constructed by using Fast Minimum Evolution method (Desper and Gascuel, 2004) in which the *Cytb* gene sequences of the domestic pigeon was compared with all the available *Cytb* gene sequences of *Columbiformes* (pigeons and doves). The rooted tree was formed by placing a root in the middle of the longest edge (Desper and Gascuel, 2004). The Fast Minimum Evolution uses the combination of greedy construction (GME) and the balanced nearest neighbor interchanges (BNNI) algorithms (Desper and Gascuel, 2004).

RESULTS AND DISCUSSION

In this study, the complete *Cytb* gene (1143 bases) of Pakistani domestic pigeon; *Sherazi* was sequenced. Homology analysis divulged 5 novel single nucleotide polymorphic sites in *Cytb* gene of the Pakistani domestic pigeon (Table 1). The *Cytb* gene sequences of the Pakistani domestic pigeon were released under the accession No. JX968124-JX968148. The overall nucleotide diversity observed in the domestic pigeon and rock pigeon was 0.6%. Mutations have strong influence on mitochondrial DNA diversity patterns in birds. There is a strong relationship between species maximal longevity and mitochondrial mutation rate which is in agreement with the mitochondrial theory of ageing (Nabholz *et al.*, 2009). The mitochondrial gene sequences along with multiple nuclear genes have been used to distinguish the speciation arising from high regional selective sweeps (Baker *et al.*, 2009). The overall nucleotide diversity of 0.16 *Columba bollii* and *Columba junoniae* restricted us to deem the Pakistani domestic pigeon as an independent species (Marrero *et al.*, 2008).

The phylogenetic tree further explained the evolutionary status of the Pakistani domestic pigeon (Fig 1). The result of phylogenetic analysis, using the *Cytb* gene sequences of the Pakistani domestic pigeon with all the available *Cytb* gene sequences of *Columbiformes*,

revealed that rock pigeon *C. livia* is the ancestor of the domestic pigeon and the Hill pigeon (*Columba rupestris*) is the ancestor of both *C. livia* and the domestic pigeon. The findings are unswerving with the whole genomic studies (Shapiro *et al.*, 2013). Domestic pigeon breeds are so distinct that, based on morphology alone, a taxonomist might be tempted to classify them as completely different genera yet all breeds are simply variants within single specie, the rock pigeon *C. livia* (Stringham *et al.*, 2012). In many cases, the variations among breeds are due to more macro-evolutionary changes than the changes within a single species (Shapiro *et al.*, 2013). As with many domesticated species, the evolution of pigeon breeds is probably not exclusively linear or hierarchical (Shapiro *et al.*, 2013; Stringham *et al.*, 2012), therefore, a new non-classical classification system is needed to reconcile the taxonomy of the domestic pigeons considering the genetic pattern of the pigeon species and breeds. The pigeon is underused as a genetic model to study the avian variation because of the rarity of genetic and genomic resources for this bird but the pigeons are a model for identifying the genetic basis of variation in traits of general interest (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 *Cytb* gene of the Pakistani domestic pigeon and its phylogeny.

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