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PHYLOGENETIC ANALYSIS OF *CAPRA HIRCUS* COMMONLY FOUND GOAT BREEDS OF PAKISTAN USING DNA BARCODE

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ABSTRACT

Cytochrome c oxidase subunit I or COI in the mitochondrial genome (DNA Barcode) of goats was used to identify and differentiate two common breeds (beetal and berberi) and crossbreeds sampled in Punjab, Pakistan. This is the first study on the molecular taxonomy of the goat breeds of Pakistan. Sequencing of DNA barcode of the beetal goat showed a 99% similarity to *Capra hircus* isolate LS16 cytochrome c oxidase subunit 1 (CO1) gene. Beriberi goats showed a 99% similarity to *Capra hircus* breed Jining Qing goat mitochondrion. Identification of goat breeds via DNA barcoding may help in local genetic improvement and conservation programs, especially in pre-screening important breeds that can be considered for conservation in their pure form. However, more COI sequences should be determined from the native goat populations of Pakistan to improve reliability of using DNA barcodes to differentiate them from their exotic counterparts. Thus, the present study concluded that DNA barcoding can be used to confirm the species or breed origin of an unknown specimen and it is a reliable and practical tool to protect the local biodiversity of livestock genetic resources. Our results validated the effectiveness of barcoding for the identification of goat breeds.

Keywords: COI gene, *Capra hircus*, livestock, Molecular identification.

INTRODUCTION

Pakistan, being an agricultural land, shares a huge amount of the livestock industry by contributing 11.8% of the national Gross Domestic Product (GDP) with the large number of domesticated animals. Goats share this GDP with a population of 68.4 Million, producing 845,000 tonnes of milk production, and 3,696,000 tonnes total meat production, as well as 26,359 million of skin products (Pakistan Economic Survey 2014-2015). It's believed that the domesticated goat (*Capra hircus*) shares an inheritance from two species of wild goat (*aegagrus* and *falconeri*) (Tu, 1989).

Losing the biodiversity, and the excessive crossbreeding, led to the species losing their identities. The Conservative Group on International Agriculture Research (CGIAR) argues that losing these breeds

weakens the genetic diversity in our food supply (FAO, 2007).. DNA Barcoding emphasizes the demarcation of species as it provides an overview of goat evolution and the genetic connection of goat breeds with other populations (Hajibabaei *et al.*, 2007). The present study was carried out to identify and generate the DNA barcodes of two commonly found goat breeds of Pakistan.

MATERIALS AND METHOD

Blood samples were collected from the Jugular veins of 30 goats, after cleaning the surface with a Pyodine solution. Disposable syringes were used. Blood was transferred to the EDTA containing vacutainers to prevent the blood from coagulation. DNA Extraction was carried out from the blood samples by following the procedure described by Sambrook and Russel, 2001.

The DNA barcode region (approximately 700 bp) of the COI gene was amplified by using two different universal primers pair Vf1d/ Vr1d and Zlr04/Zlf04. Details of the primers are given in Table 1. The 25 µL PCR reaction mixture was prepared containing 2.5µL of 10X PCR buffer, 2.5 µL of 25 mM MgCl₂, 0.2 mM each dNTP, 1.25 µL of 10 µM each primer, 2.5 U of Taq polymerase, 100ng of DNA template, and PCR water. Amplification was done following PCR cycling protocol (pre-denaturation at 95 °C for 5 min, 35 cycles of 95 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min, post extension at 72°C for 5 min) and the PCR products were visualized and analyzed on 1.5% agarose gel. The amplicons were sequenced by the Sangers Method (Sanger *et al.*, 1977). The obtained sequences

(Chromatograms: Supplementary Material 1, Sequence FASTA Format: Supplementary Material 2), were aligned using NCBI's BLAST

(<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

RESULTS AND DISCUSSION

The approximately 700 bp barcode amplicons (Figure 1) sequenced from all the samples of the goats showed a 99% similarity with *Capra hircus* isolate LS16 cytochrome c oxidase subunit 1 (CO1) gene and Capra hircus breed Jining Qing goat mitochondrion, complete genome; Accession No. gb|KJ920218.1 and gb|KP677510. Migration of the people, trade between the people, and cross-breeding are the reasons for the emergence of exotic breeds in Pakistan.

Table 1: Properties of primers pair used for DNA barcoding of goats.

No	Primer	Primer Sequence (5' to 3')	Ta (°C)	Product size (bp)	Reference
1	Z104 (F)	TCT CAA CTA AYC AYA AAG AYA TYG G	50	650	Tavares <i>et al.</i> , 2008
	Z104 (R)	TAA ACT TCR GGG TGA CCA AAR AAT CA			
2	VF1d (F)	TTC TCA ACC AAC CAC AAR GAY ATY GG	50	750	Tavares <i>et al.</i> , 2008
	VF1d (R)	TAG ACT TCT GGG TGG CCR AAR AAY CA			

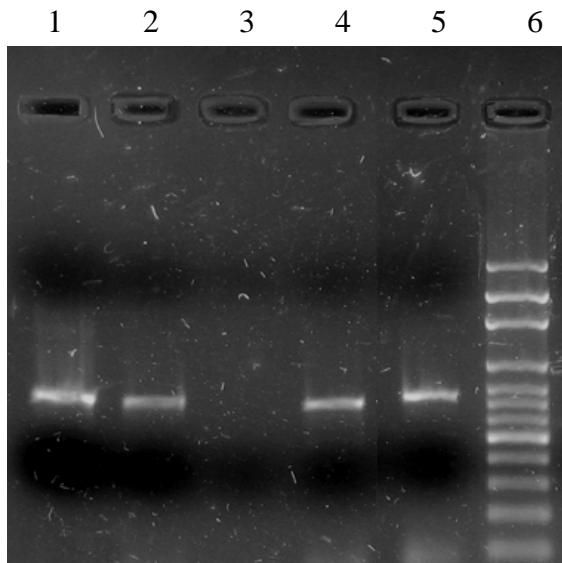


Figure 1: PCR gel of folmer region of CO I gene amplified from the goat

samples. Lane 1, 2 and 4: 650 bp PCR product of beetal goat breed samples, Lane 5: 750 bp PCR products of berberi goat samples and Lane L: 100bp plus DNA ladder (Fermentas, USA).

The present study revealed that the sequence showed a maximum homology (99%) with black goats (*Capra hircus*) and also generated DNA barcodes successfully. Many previous studies highlighted the difficulties in acquiring DNA barcodes data, like the high variability of priming sites in vertebrates (Clare *et al.*, 2007 Vences *et al.*, 2005). Thus, we used two sets of cocktail primers, among which vf1d/vr1d showed more successful results. The present study

reaffirmed the effectiveness of the barcoding of goat breeds of Pakistan.

Our results suggested that DNA barcodes are a highly effective identification system for the goat breeds of Pakistan. Adding barcode sequences in a database with primer sequences and related quality scores, will make it widely reachable. The assembly of a DNA barcode library for livestock will not only help in species identification, but will also result in the development of an automated identification system, which will be valuable for law enforcement and allow conservation officials to identify poachers and smugglers. However, the present study only analyzed goat breeds, and the specimens were mainly collected in Punjab, Pakistan. Additional studies, all-inclusive taxonomic samples, as well as populations from other geographical regions, are required.

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