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IDENTIFICATION OF GOAT SPECIE IN DISTRICT SARGODHA BY USING CO-ENZYME I FROM SARGODHA DISTRICT, PUNJAB

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Abstract
This research focuses on identifying goat species in the Sargodha District through the application of Coenzyme I, a vital element in numerous metabolic pathways. The primary goal is to investigate genetic diversity among local goat breeds, which is crucial for the development of effective breeding and conservation strategies. Molecular techniques were employed to collect DNA samples from multiple goat populations within the district. Coenzyme I was used as a biochemical marker in the analysis, facilitating the differentiation of species based on genetic profiles. The results revealed distinct genetic patterns associated with environmental adaptations and specific phenotypic traits. This study underscores the potential of Coenzyme I in distinguishing goat species and provides a detailed view of the genetic landscape of goat populations in Sargodha. Moreover, the findings contribute to the broader understanding of livestock genetics by illustrating the significance of molecular markers in evaluating genetic variation. This information is instrumental in guiding breeding programs that aim to enhance desirable traits such as disease resistance, productivity, and environmental adaptability. In conclusion, the study highlights the value of integrating biochemical markers like Coenzyme I into livestock management practices, ultimately supporting the advancement of goat husbandry in the region.

INTRODUCTION

The identification of goat species in District Sargodha is a task of immense importance, with widespread implications for animal husbandry, environmental sustainability, rural development, and public health. In this region, goats are more than just livestock—they are a cornerstone of agricultural livelihoods, providing essential resources such as milk, meat, fiber, and even manure for crop farming. In many rural households, especially those with limited land or income, goats serve as both a source of nutrition and financial security [4]. Their adaptability to harsh climates, scarce water, and minimal feed availability makes them particularly valuable in regions like Sargodha, where resources can be limited [5].

Despite their significance, the accurate identification and classification of goat breeds in the region is still a pressing challenge. The diversity of the goat population, combined with limited access to detailed morphological and genetic data, often leads to confusion and misidentification among local breeds [6]. Such inaccuracies can have serious consequences. Misidentification may result in poorly managed

breeding programs that dilute the genetic purity of superior breeds, lower resistance to diseases, and reduce productivity traits such as milk yield or meat quality. For instance, indigenous breeds like Beetal, known for their excellent milk production, and Kamori, prized for high-quality meat, could be wrongly categorized or undervalued without proper identification methods [7].

In response to these challenges, researchers have increasingly turned to advanced morphometric analysis and molecular genetics as tools for breed differentiation. Morphometric analysis involves the systematic measurement of physical traits—such as ear length, body size, and horn shape— while genetic tools use DNA markers to detect genetic variation and lineage. Together, these techniques provide a reliable foundation for identifying goat species with a high degree of accuracy [8]. This accurate classification critical not only for breed conservation but also for the development of targeted breeding programs that can enhance productivity and resilience.

The benefits of precise species identification go beyond individual farm productivity—it plays a pivotal role in biodiversity conservation. Indigenous goat breeds possess unique genetic traits that enable them to survive and thrive in specific environments. These adaptive traits, such as tolerance to local parasites or the ability to produce milk under heat stress, are crucial for sustainable agriculture, especially in the face of climate change [9]. If these local breeds are not properly recognized and protected, they could be lost through crossbreeding or replacement by exotic breeds, leading to a decline in genetic diversity and the erosion of valuable traits. Conservation of these breeds also preserves cultural heritage, as many goat breeds are historically linked to regional traditions, social customs, and ceremonies [5].

Health management is another domain where species-specific knowledge proves essential. Certain diseases, like fascioliasis—a parasitic infection common in Pakistan's rural areas— disproportionately affect specific breeds due to their genetic makeup or feeding habits [10]. If health interventions are designed without considering these breed-specific vulnerabilities, their effectiveness may be compromised. Accurate identification allows veterinarians and policymakers to design precise treatment, vaccination, and prevention strategies tailored to the biological traits of each breed, reducing animal mortality and improving herd health outcomes.

From an economic perspective, better breed identification allows farmers to strategically select goats based on desired traits, improving market alignment and income generation. For example, breeds such as Saanen and Nubian are known worldwide for their high milk yield, while breeds like Kamori are favored for meat production due to their fast growth and high-quality carcass yield [11]. When farmers can accurately identify and breed for these traits, they can enhance both the quantity and quality of their production, leading to better market prices and increased household income. Additionally, value-added products like goat cheese, butter, and yogurt are gaining popularity in both domestic and international markets. Identifying the best dairy breeds can help rural communities tap into these markets and diversify their income sources [12].

Public health concerns further elevate the importance of species identification. Goats are known carriers of zoonotic diseases—illnesses that can transfer from animals to humans. These include tick-borne fevers, brucellosis, and gastrointestinal parasites. Different breeds show varying levels of susceptibility to such diseases, which means that a one-size-fits-all health approach is insufficient [13]. Understanding which breeds are more vulnerable can lead to more efficient resource allocation for disease control, reducing the risks to both animal and human populations in rural areas. Moreover, this knowledge supports the development of early warning systems and targeted biosecurity measures that can mitigate the spread of zoonotic infections. The primary objectives of this research study are to identify and differentiate goat species in the Sargodha District using Coenzyme I as a molecular marker, assess the genetic diversity among local goat populations to understand their environmental adaptations and phenotypic traits, and contribute to the development of effective breeding and conservation strategies by integrating the molecular data generated into livestock management practices. The insights gained from this molecular analysis can then be used to inform breeding programs and conservation efforts, ensuring the sustainable

management of these valuable livestock resources while preserving their unique environmental adaptations and desirable phenotypic characteristics.

MATERIALS AND METHODS

In this study, blood samples were collected from different goat populations across District Sargodha, Pakistan. The goal was to identify species using the CO1 gene—a reliable genetic marker used in modern molecular techniques. Since DNA can degrade easily if not handled properly, the samples were collected in EDTA tubes, which help preserve the blood, and were kept at cold temperatures until they could be processed in the lab.

To avoid any contamination, extra care was taken during sample collection. All tools and containers were thoroughly sterilized, and strict hygiene protocols were followed throughout the process to ensure the accuracy of the results. Using the CO1 gene for barcoding has several clear advantages over traditional identification methods. It's objective and consistent, so there's no guesswork involved.

Overall, this approach provides a modern, efficient, and highly accurate way to identify goat species and understand their genetic differences—something that's especially useful in regions like Sargodha where breed distinctions are often unclear just by looking at the animals.

Sampling Detail

Collection of samples from different region of Sargodha

SR.NO	SAMPLE NAME	SAMPLE ID	COLLECTION SITE	COLLECTI ON DATE
01	*Capra hircus*	SAMPLE ID 01	Shaheen abad	11-11-2024
02	*Capra hircus*	SAMPLE ID 02	Iqbal Colony	12-11-2024
03	*Capra hircus*	SAMPLE ID 03	Sargodha city	26-11-2024
04	*Capra hircus*	SAMPLE ID 04	Chandi chok	28-11-2024
05	*Capra hircus*	SAMPLE ID 06	Satellite Town	11.12.2024

DNA Extraction from Meat Samples

To extract DNA from goat meat, a reliable method was used that combines proteinase K digestion with phenol-chloroform extraction. This approach is known to give both high-quality and high-yield DNA, especially from muscle tissue.

Sample Preparation

- Take approximately 25–30 mg of goat meat.
- Finely mince the meat and place it into a clean Eppendorf tube.

Cell Lysis

- Add lysis buffer (containing sucrose, Tris-HCl, $MgCl_2$, and Triton X-100) to the sample.
- Add 40 µl of SDS to help break down the cell membranes.

Protein Digestion

- Add 20 µl of Proteinase K to the mixture.
- Incubate the tube overnight at 56°C to allow complete digestion of proteins.

Centrifugation

- After incubation, centrifuge the mixture at 13,000 rpm for 8 minutes.
- This step separates the cell debris from the DNA-containing solution.

DNA Purification

- Transfer the supernatant to a new tube.
- Add an equal volume of phenol:chloroform:isoamyl alcohol solution (25:24:1).
- Mix gently, then centrifuge again to separate the layers.

DNA Collection

- Carefully collect the top aqueous layer (which contains the DNA).
- Proceed with ethanol precipitation or another final clean-up method as needed.

Storage

- Store the purified DNA at -20°C until ready for PCR or further analysis.

PCR Amplification of the COI Gene

Once the DNA was ready, a specific gene known as COI was targeted using a technique called PCR (polymerase chain reaction). This method works like a molecular photocopier, making millions of copies of the COI gene so that it can be easily studied.

Universal primers, which are short DNA sequences that bind to the start and end of the COI gene, were used to kick off the process. The PCR conditions included:

- An initial heating step to open up the DNA strands.
- 35 cycles of:
  - Heating to separate the DNA strands,
  - Cooling to let the primers attach,
  - And warming to build new strands of DNA.
- A final step ensured that all the copies were fully completed.

Sequencing and Species Identification

After the COI gene was amplified, the resulting DNA fragments were sent for Sanger sequencing—a precise and well-established method for reading the DNA code.

Once the DNA sequences were obtained, they were compared to known sequences stored in global databases like GenBank (NCBI). These comparisons allowed researchers to confirm which species or breed the meat samples came from. If the sequence matched a known reference with 98–99% or higher accuracy, it confirmed the identity of the goat species.

RESULTS

DNA Extraction and Quantification

Genomic DNA was efficiently extracted from five goat meat samples using a modified phenol-chloroform method, based on the protocol by Sambrook and Russell, with enhancements such as proteinase K digestion to improve tissue lysis and DNA yield [30]. The integrity and purity of the DNA were verified through 1% agarose gel electrophoresis, which confirmed the presence of intact genomic DNA and the absence of significant degradation or RNA contamination. Accurate quantification followed, ensuring that the DNA was of sufficient quality and concentration for subsequent molecular procedures. High-purity DNA is essential for effective PCR amplification, as contaminants can inhibit enzymatic processes and compromise sequencing results [31].

Polymerase Chain Reaction (PCR)

Following DNA quality assessment, the mitochondrial cytochrome c oxidase subunit I (CO1) gene was amplified using polymerase chain reaction (PCR), which is widely used for species identification due to the gene's conserved and variable regions [32]. Universal CO1 primers targeting a ~650 bp fragment were applied, with reaction parameters optimized to enhance amplification specificity and efficiency across different goat breeds. Conditions such as annealing temperature and cycle number were carefully adjusted to reduce nonspecific amplification. The amplified products were confirmed through gel electrophoresis to ensure successful CO1 gene amplification before sequencing was conducted [33].

Maximum Likelihood Estimate of Substitution Matrix

	A	T/U	C	G
A	-	*5.61*	*5.82*	**13.02**
T/U	*5.95*	-	**13.81**	*5.51*
C	*5.95*	**13.32**	-	*5.51*
G	**14.06**	*5.61*	*5.82*	-

NOTE.– Each entry is the probability of substitution (r) from one base (row) to another base (column). Substitution pattern and rates were estimated under the Tamura-Nei (1993) model [1]. Rates of different transitional substitutions are shown in **bold** and those of transversionsal substitutions are shown in *italics*. Relative values of instantaneous r should be considered when evaluating them. For simplicity, sum

of *r* values is made equal to 100, The nucleotide frequencies are A = 26.01%, T/U = 24.51%, C = 25.42%, and G = 24.07%. For estimating ML values, a tree topology was automatically computed. The maximum Log likelihood for this computation was -14690.948. This analysis involved 18 nucleotide sequences. There were a total of 1451 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [2]

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Maximum Likelihood Estimate of Transition/Transversion Bias

FROM\TO	A	T	C	G
A	-	5.7395	5.7395	13.5209
T	5.7395	-	13.5209	5.7395
C	5.7395	13.5209	-	5.7395
G	13.5209	5.7395	5.7395	-

The estimated Transition/Transversion bias (*R*) is 1.18. Substitution pattern and rates were estimated under the Kimura (1980) 2-parameter model [1]. The nucleotide frequencies are A = 25.00%, T/U = 25.00%, C = 25.00%, and G
= 25.00%. For estimating ML values, a tree topology was automatically computed. The maximum Log likelihood for this computation was -14689.462. This analysis involved 18 nucleotide sequences. There were a total of 1451 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [2]

DISCUSSION AND CONCLUSION

The application of genetic markers has become an essential approach in assessing biodiversity, tracing evolutionary relationships, and guiding the effective management of domesticated animal populations. Among various genetic markers, the mitochondrial Cytochrome c Oxidase I (COI) gene has emerged as a key tool for precise species identification and phylogenetic studies, particularly in livestock species such as *Capra hircus* (domestic goat) [36]. The COI gene facilitates the examination of genetic diversity, population structure, and lineage differentiation—critical aspects in regions like District Sargodha, where a wide variety of indigenous and hybrid goat breeds are raised under different farming systems.

Located in the Punjab province of Pakistan, Sargodha represents a region of significant livestock diversity, especially in goats. These animals play a central role in supporting rural livelihoods through the provision of meat, milk, and hides. This has highlighted the necessity of molecular genetic tools, particularly mitochondrial DNA markers such as COI, to resolve these complexities and support systematic breed classification [37]. when analyzing goat populations in Sargodha, where visual similarities often obscure underlying genetic differences. COI-based analysis allows for the detection of cryptic variation and identification of unique genetic signatures, which are essential for validating breed identity and ensuring the conservation of valuable genetic resources [38].In global research, COI barcoding has been effectively used to differentiate goat breeds and investigate their phylogenetic relationships. Applying the same method to goat populations in Sargodha can reveal whether they belong to distinct genetic lineages or represent hybrids formed through recent gene flow. Such insights are vital in areas where traditional breed classification
systems may not reflect the true genetic structure of the population [39].

Moreover, the resolution provided by the COI gene enables the construction of phylogenetic trees, helping

to trace the evolutionary history and maternal lineage dispersal within local goat populations. By aligning COI sequences from Sargodha's goats with reference data in international databases such as GenBank and the Barcode of Life Data System (BOLD), researchers can situate these populations within a broader evolutionary and geographic context. This helps to reconstruct domestication routes and identify historical breeding patterns, offering insights into how breeds have evolved over time [40].

From a conservation perspective, COI gene sequencing offers valuable guidance for identifying and prioritizing genetically distinct goat populations. Conservation programs can focus on preserving lineages that exhibit unique COI profiles, which may also confer adaptive advantages in local environments. This genetic information is essential for supporting the sustainable utilization and long-term preservation of goat breeds in Sargodha, contributing to food security, cultural heritage, and resilience to environmental challenges [41]. While other mitochondrial markers like Cytochrome b (CYTB) are also used in genetic studies, COI provides several advantages, including higher resolution and a more comprehensive reference library in global databases. The wide availability of COI sequences ensures accurate identification and enhances comparative studies across populations and regions [42].

This study highlights the effectiveness of the cytochrome c oxidase subunit I (COI) gene as a molecular marker for evaluating the genetic diversity of *Capra hircus* in District Sargodha, where traditional phenotypic classification methods are limited by breed similarity and genetic admixture [41]. The maternally inherited and highly mutable nature of the COI gene makes it particularly valuable for tracing maternal lineages and detecting subtle genetic variations, enabling better-informed breeding decisions that promote sustainability. Additionally, phylogenetic analyses based on COI sequences provide insights into the evolutionary history and domestication patterns of local goats, while comparisons with global genetic databases help verify species identity and assess the uniqueness of Sargodha's goat populations in an international context [40]. Overall, COI barcoding proves to be a reliable tool for species identification, conservation planning, and improving livestock practices. Future studies should broaden genetic sampling and integrate additional molecular markers to develop a more comprehensive genetic profile of *Capra hircus* in the region.

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FIGURES

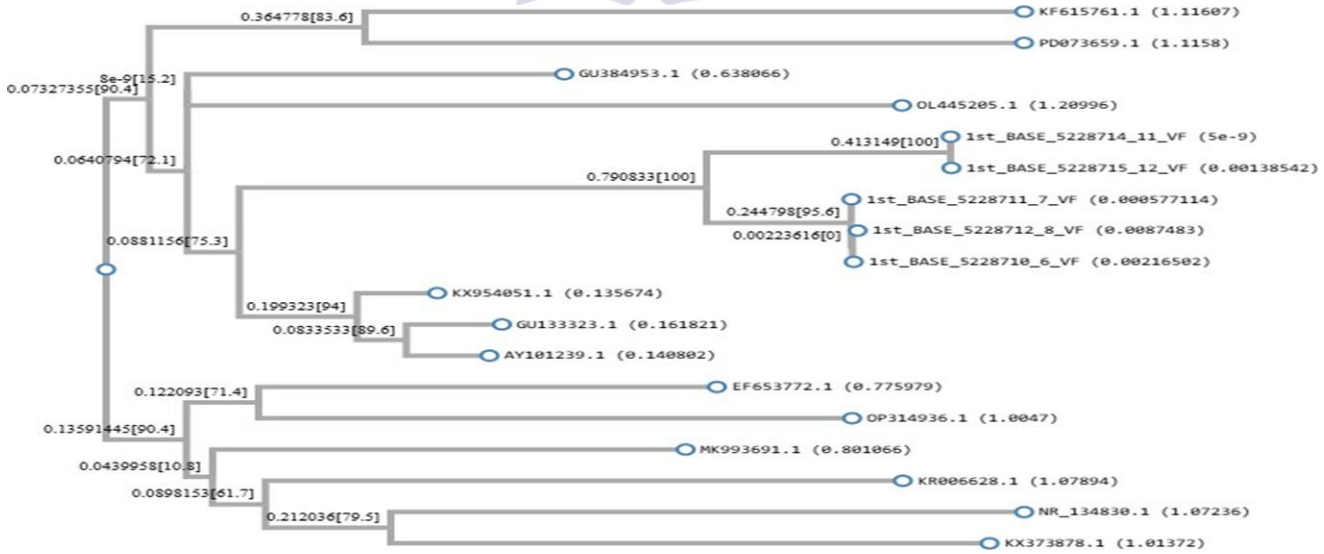

Fig 1: Phylogenetic analysis pipeline by ETE3Workflow Phylogenetic analysis pipeline by ETE3Workflow

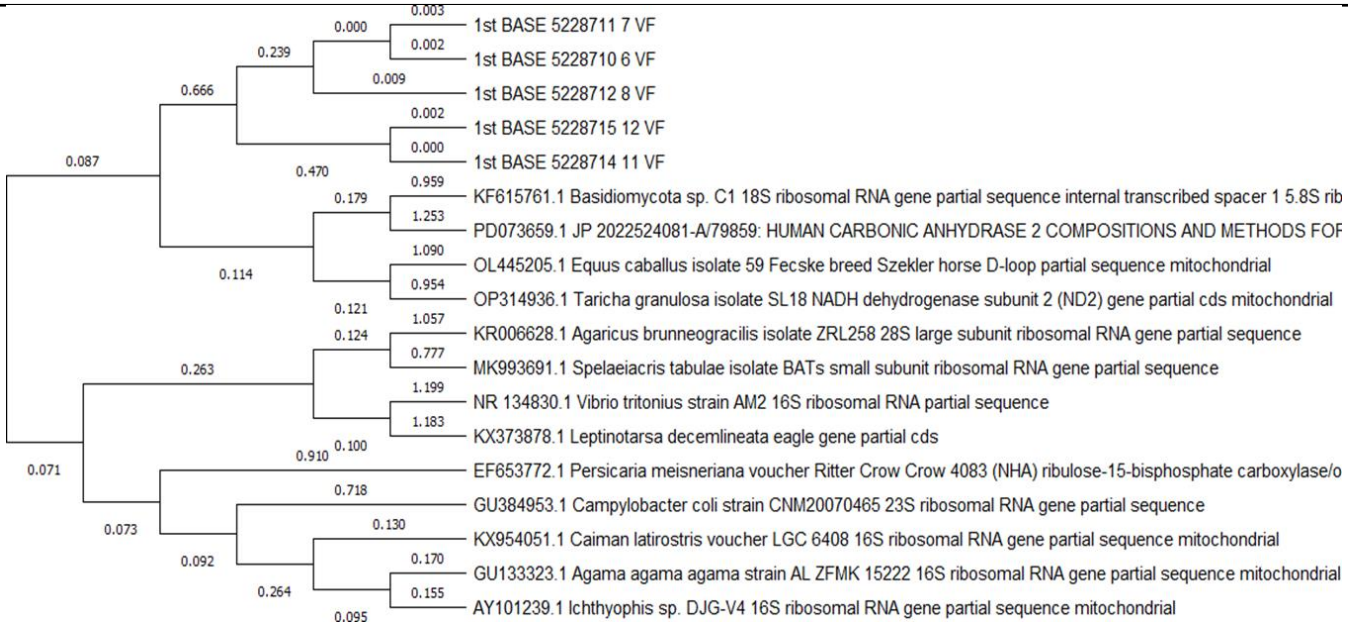

Fig 2: Maximum Likelihood method and Tamura-Nei model

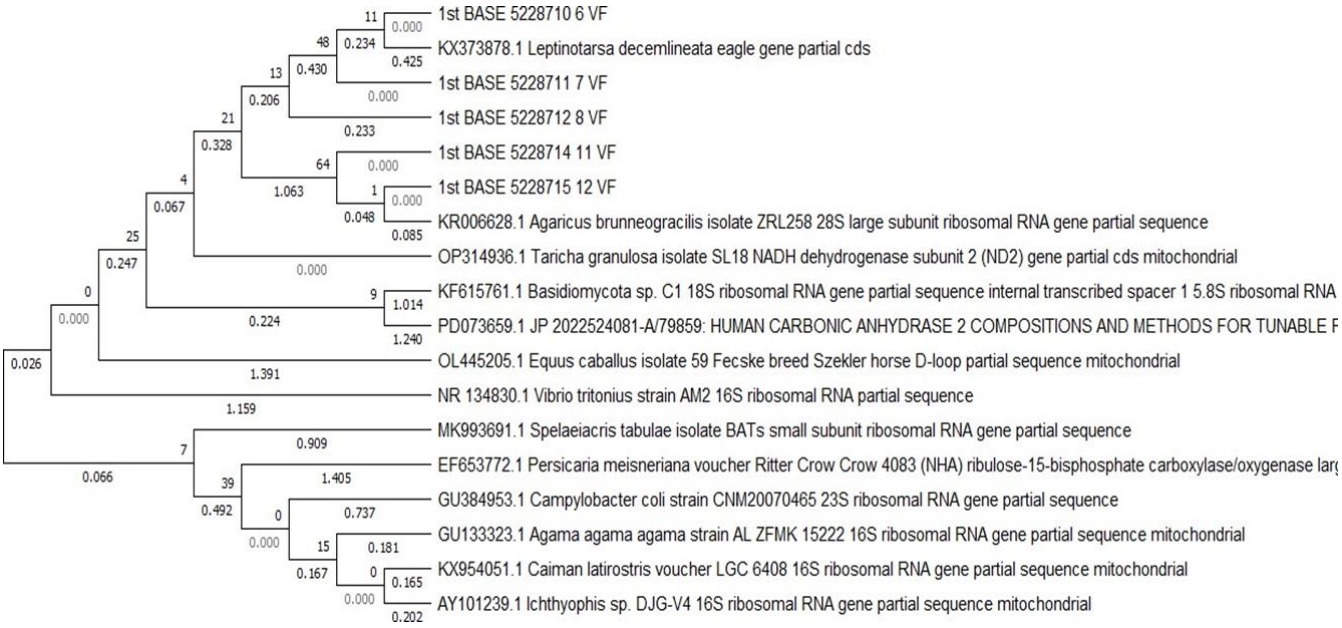

Fig 3: Neighbor-Joining method