Pakistan J. Zool., vol. 52(2), pp 441-446, 2020. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20190822100843

Assessment of Mitochondrial *ATPase 8/6* Genes Based Genetic Diversity in Sindh Ibex (*Capra aegagrus* Blythi)

Javeria Zafar[1], Asif Nadeem[1]*, Maryam Javed[1], Fehmeeda Fatima[1], Wasim Shehzad[1], Ghulam Abbas[1], Rajput Zahid Iqbal[2] and Muhammad Muddassir Ali[1]

[1]*Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences Lahore*
[2]*Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand*

JZ and AN made equal contributions to this article.

Article Information
Received 22 August 2019
Revised 01 September 2019
Accepted 07 September 2019
Available online 16 January 2020

Authors' Contribution
AN designed the study. JZ and AN carried out the genomic work. GA and RZI collected the samples. MJ, FF and MMA analyzed the data and wrote the manuscript. AN and WS revised the manuscript.

Key words
ATP synthase, Phylogenetic relationship, Parsimony analysis, Genetic diversity

ABSTRACT

The current study was designed to characterize mitochondrial *ATPase8/6* genes in Sindh ibex and to determin its phylogenetic relationship with other capra species. Blood samples were collected from Kirthar National Park, Sindh, Pakistan. PCR product was sequenced bi-directionally using dideoxy chain termination method after mitochondrial *ATPase8/6* genes amplification. Polymorphism, Genetic diversity and Phylogenetic analysis were carried out by the MUSCLE, DnaSP and MEGA6 tools. Total of 20 variations at different positions were found in aligned sequence results. Sequence conservation was observed for the ibex population. The overall results showed a close evolutionary relationship among *Capra aegagrus, Capra nubiana, Capra falconeri, Capra hircus and Capra caucasica*, demonstrating that they all were evolved from same ancestor.

INTRODUCTION

Wild goat species live at mountains and at higher altitudes, may face different environmental stress according to their habitat. Moreover, domestication of these species led to the decrease in genetic variety due to increase inbreeding and hence might causes the extinction of many novel and diverse breeds.

Pakistan is fairly rich in biological diversity range and has been conventionally known for its abundance in wildlife, mainly game animals such as Bradford urial (*Ovisorientalis),* Sindh ibex or Sara (*Capra aegagrus blythi)* and Chinkara (*Gazella benneti*) (Jacobson *et al*., 2003). The estimated Sindh ibex population is almost 13,155 and densely present on the Kirthar Range, with lesser concentration on Khambu and Dumber and small numbers somewhere else. Higher altitude appear critical to this species (Sultana and Mannen, 2003). Till now many studies have been done on goat using mitochondrial DNA (mt-DNA) and of nuclear DNA (Kibegwa *et al*., 2016; Kadowaki *et al*., 2016) but no data has been published on phylogenetic diversity and genetic characterization of Sindh ibex except Heat Shock Protein 70-1 as reported by Fatima *et al*. (2019).

Mitochondrial *ATPase-8* and *ATPase-6* genes (*mtATPase 8/6*) are considered as one of the most potential genes for the whole mitochondrial genome due to its structure and function, and it is consisted of both conserved and rapidly evolving regions which are considered as more reliable regions for evolutionary studies (Farias *et al*., 2001). It is considered as one of the most valuable genetic marker to identify the relation within families and genera. (Parson *et al*., 2000). Any mutation in *ATPase 6* gene causes the abrasion in single structure, tissue or organ (e.g., myopathies, encephlopathies and cardiopathies) to multisystem syndromes (Stewart *et al*., 2009). So, in the present study, the sequencing of the whole *mtATPase 8/6* genes of Sindh ibex has been done. The variants were identified in *mtATPase 8/6* genes in Sindh ibex and phylogenetic tree was constructed to understand the genetic diversity of Sindh ibex.

MATERIALS AND METHODS

Sample collection and storage

Blood samples (n=15) of wild goat (*Capra aegagrus*

* Corresponding author: asifnadeem@uvas.edu.pk
0030-9923/2020/0002-0441 $ 9.00/0

blythi) were collected from Kirthar National Park, Sindh, Pakistan in ethylenediamine tetra acetic acid (EDTA) coated vacutainers. Samples were stored at 20^{0}C before the extraction of DNA.

Genomic DNA extraction

The blood samples were thawed and subjected to DNA extraction through organic extraction method for whole genome. Quantification of DNA was done through gel electrophoresis (0.8% agarose gel) and spectrophotometric analysis (Thermo scientific 2000 nano spectrophotometer). The gel was run at 110 volts for 30 min. BIO-RAD Gel Documentation system was used to visualize DNA bands.

PCR optimization

The PCR conditions for the amplification of *mtATPase8/6* genes were optimized by using different concentration of MgCl2, dNTPs (deoxynucleotide triphosphate) and *Taq* polymerase to get the maximum amplification of the required product.

Touchdown PCR (Bio-Rad thermo cycler) was used to optimize the primers and PCR was performed.

Sequencing of PCR amplicon

PCR amplicons were precipitated with 100µl of 80% ethanol and then sequenced in both directions using dideoxy chain termination method by Big Dye™ Terminator on ABI 3130XL Genetic analyzer.

Bioinformatics analysis

Sequences were analyzed manually by using BioEdit software V.7.0. Sequencing homology search in public databases was carried out through Nucleotide blast program (Altschul *et al*., 1990). Polymorphism was studies by the multiple sequence alignment using ClustalW2 and DnaSP, v.5 (Librado and Rozas, 2009). Any change in the DNA sequence was confirmed by sequencing both sense and antisense strands. Molecular Evolutionary Genetics Analysis V.6.0 (Tamura *et al*., 2013) was used to construct he phylogenetic trees. Percentage similarity was calculated by using the MUSCLE tool.

RESULTS AND DISCUSSION

Wild life is a vital part of ecosystem and important assets of a country. However, very few studies have been carried out on genomic and proteomic parameters of Pakistan's wild animals.

Genetic characterization of Sindh ibex was done on the basis of *mtATPase8/6* gene. For his, the amplicon of 990bp size were amplified using the PCR. Afterwords, the products of PCR were sequenced and Chromatogram of all sequenced samples were analysed.

The results showed 20 variations at different positions. All variations were found to be homozygous Transition mutations. Results of polymorphic sites, Haplotype Diversity, G+C and Tajima's test *ATPase 8/6* gene in Sindh Ibex are shown in Tables I, II, III, IV. Twenty transitional mutations were observed, in which two were singleton variable sites with two variants positioned (Table I), while rest of eighteen variations were parsimony variable sites with two variants (Table II). Average number of nucleotide differences was 7.408, whereas stochastic variance of k (no recombination), Vst (k) was calculated as 11.695 and G+C contents in whole population of Sindh ibex were 0.361 (519.00 sites).

Table I. Polymorphic sites of *ATPase 8/6* gene in Sindh ibex.

Number of sequences	15
Number of sequences used	15
Selected region	1-973
Number of sites	973
Total number of sites (excluding sites with gaps / missing data	519
Sites with alignment gaps or missing data	454
Invariable (monomorphic) sites	501
Variable (polymorphic) sites	20
Total number of mutations	20
Singleton variable sites	2
Parsimony informative sites	18
Singleton variable sites (two variants)	2
Site positions	193, 505
Parsimony informative sites (two variants)	18
Site positions	120, 133, 162, 183,185, 187, 201, 209, 220, 223, 291, 391, 418, 441, 448, 468, 509, 517

Conserved region identification showed the sequence conservation for the ibex population, 0.967 and the conservation threshold for population was 1. Conserved region for ibex specie was from 518-653 bp, comprising 135 nucleotides as shown in Table V.

Furthermpre, the results of homology analysis indicated that partial sequence of *ATPase8/6* genes of Sindh ibex showed maximum similarity (99%) with that of domestic goat and sheep.

Table II. Haplotype diversity of *ATPase 8/6* gene in Sindh ibex.

Number of Haplotype, h	5
Haplotype (gene) diversity, Hd	0.0808
Variance of Haplotype diversity	0.00277
Standard Deviation of Haplotype diversity	0.053
Nucleotide diversity, Pi	0.01427
Theta (per site) from Eta	0.01045
Theta (per site) from S, Theta-W	0.01045
Variance of theta (no recombination)	0.0000190
Standard deviation of theta (no recombination)	0.00436
Variance of theta (free recombination)	0.0000061
Standard deviation of theta (free recombination)	0.00246
Average number of nucleotide differences, k	7.408
Stochastic variance of k (no recombination), Vst(k)	11.695
Sampling variance of k (no recombination), Vs(k)	1.675
Total variance of k (no recombination), V(k)	13.369
Stochastic variance of k (free recombination), Vst(k)	2.469
Sampling variance of k (free recombination), Vs(k)	0.329
Total variance of k (free recombination), V(k)	2.799
Variance of theta (no recombination)	5.123
Variance of theta (free recombination)	1.635

Table III. G+C Contents of *ATPase 8/6* gene in Sindh ibex.

G+C content,	
G+C	0.361 (519.00 sites)

Table IV. Tajima's test and Tajima's test of *ATPase 8/6* gene in Sindh ibex.

Tajima's D	1.45787
Statistical significance	Not significant, P > 0.10
Fu and Li's D* test statistic	1.01041
Statistical significance	Not significant P > 0.10

Phylogenetic analysis showed two distinct clades and *ATPase8/6* gene of Sindh ibex was highly homologous to that of other animals as shown in Figues 1 and 2.

The overall results showed a close evolutionary relationship with other animals, demonstrating that they all were evolved from same ancestor. Phylogenetic analysis showed that *Capra aegagrus, Capra nubiana, Capra falconeri, Capra hircus and Capra caucasica* were sharing a common ancestors. Previous studies revealed that the bezoar, markhor and other wild goats are distantly related to the domestic goat, based on mtDNA analysis (Pidancier *et al*., 2006; Sultana *et al*., 2003).

Table V. Conserved region identification of *ATPase 8/6* gene in Sindh ibex.

Net number of analyzed sites, L	613
Number of variable/polymorphic sites S	20
Sequence conservation, C	0.967
Net number of analyzed sites, L	613
Number of variable/polymorphic sites, S	20
Conserved Region	
Conservation threshold, CT	1
[Region Start-End Conservation Homozigosity P-value] Region_1, 518-653, 1.000, 1.000,0.0061	
ATCCTATTTATTGGATCTACAAACCTATTAGGCCTTC-TACCCCACTCATT TACACCAACTACACAACTATCAATAAATCTAGGCAT-GGCTATTCCCTTAT GAGCAGGGGCTGTAATTACAGGTTTTCGCAACAAAA	

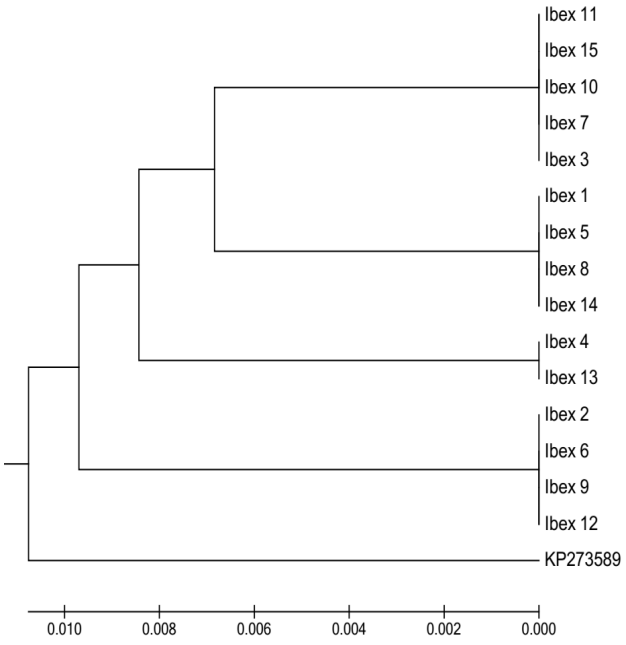

Fig. 1. UPGMA tree construction within-specie by using MEGA6.

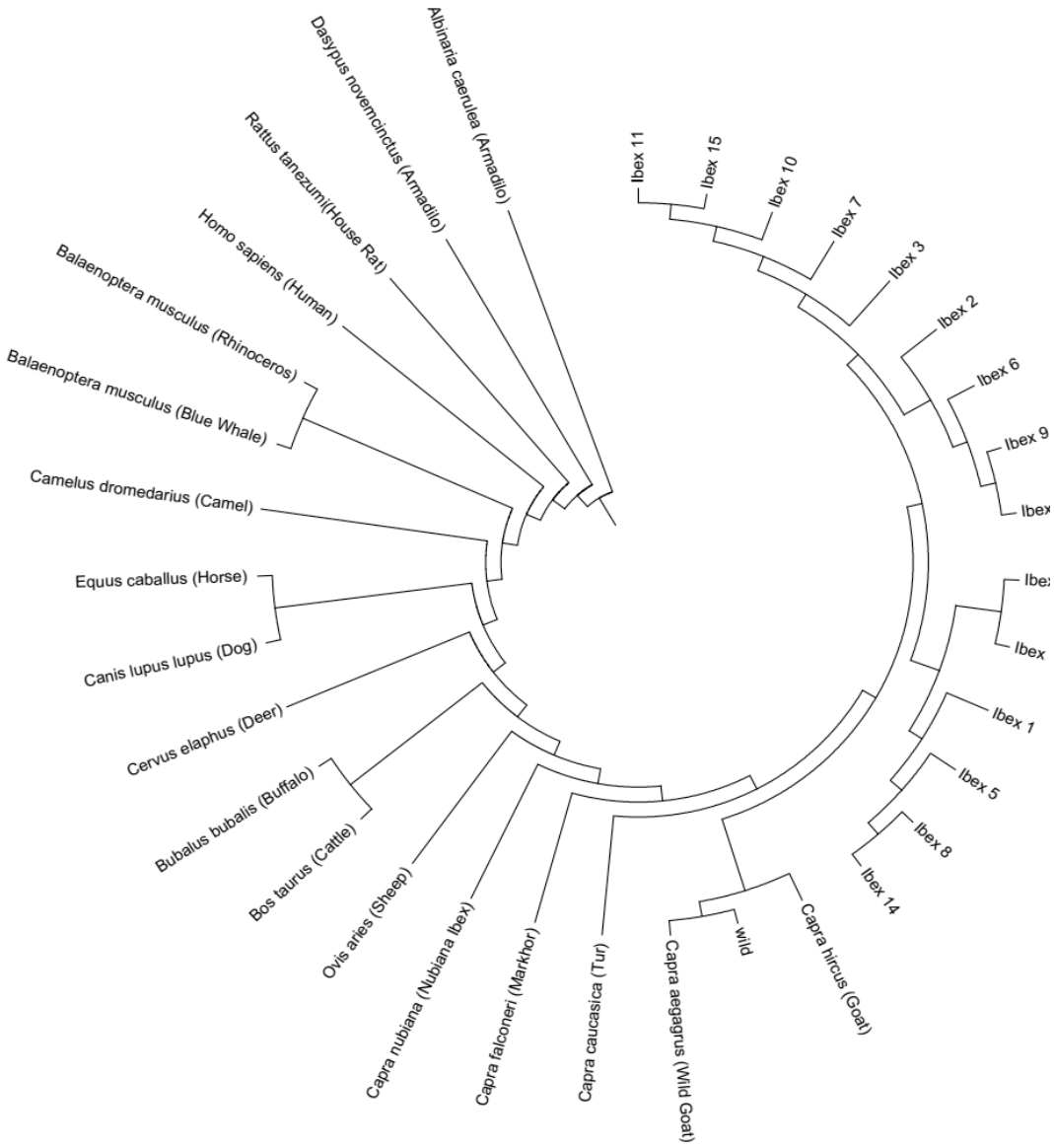

Fig. 2. UPGMA tree construction between-species by using MEGA6.

CONCLUSION

The results of the current study will help in understanding the life and evolutionary patterns of Sind Ibex. However, further molecular studies are needed by using the domestic goats from other countries, for better understanding and planning the conservation strategies of wildlife.

Statement of conflict of interests

The authors declare that they have no competing interests.

REFERENCES

Ackerman, S.H. and Tzagoloff, A., 2005. Function, structure, and biogenesis of mitochondrial ATP synthase. *Progr. Nucl. Acid Res. mol. Biol.,* **80**: 95-133. https://doi.org/10.1016/S0079-6603(05)80003-0

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J., 1990. Basic local alignment search tool. *J. mol. Biol.,* **215**: 403-410. https://doi.org/10.1016/S0022-2836(05)80360-2

Arnot, D.E., Roper, C. and Bayoumi, R.A., 1993. Digital

codes from hypervariable tandemly repeated DNA sequences in the *Plasmodium falciparum* circumsporozoite gene can genetically barcode isolates. *Mol. Biochem. Parasitol.,* **61:** 15-24. https://doi.org/10.1016/0166-6851(93)90154-P

Barendse, W., Armitage, S.M., Kirkpatrick, B.W., Moore, S.S., Georges, M., Womack, J.E. and Hetzel, J., 1993. A genetic map of index DNA loci on bovine chromosome 21. *Genomics,* **18**: 598-601. https://doi.org/10.1016/S0888-7543(05)80361-1

Boyer, P.D., 1997. The ATP synthase-a splendid molecular machine. *Annu. Rev. Biochem.,* **66**: 717-749. https://doi.org/10.1146/annurev.biochem.66.1.717

Cassidy, L.M., Teasdale, M.D., Carolan, S., Enright, R., Werner, R., Bradley, D.G., Finlay, E.K. and Mattiangeli, V., 2017. Capturing goats: documenting two hundred years of mitochondrial DNA diversity among goat populations from Britain and Ireland. *Biol. Lett.,* **13:** 20160876. https://doi.org/10.1098/rsbl.2016.0876

Castresana, J., 2001. Comparative genomics and bioenergetics. *Biochim. biophys. Acta (BBA-Bioenergetics),* **1506**: 147-162. https://doi.org/10.1016/S0005-2728(01)00227-4

Collinson, I.R., Runswick, M.J., Buchanan, S.K., Fearnley, I.M., Skehel, J.M., van Raaij, M.J. and Walker, J.E., 1994. F0 membrane domain of ATP synthase from bovine heart mitochondria: Purification, subunit composition, and reconstitution with F1-ATPase. *Biochemistry,* **33**: 7971-7978. https://doi.org/10.1021/bi00191a026

Fatima, F., Nadeem, A. and Javed, M., 2019. Molecular characterization of heat shock protein 70-1 gene of *Capra aegagrus blythi. Pakistan J. Zool.,* **51**:195-203.

Farias, I.P., Ortí, G., Sampaio, I., Schneider, H. and Meyer, A., 2001. The cytochrome b gene as a phylogenetic marker: the limits of resolution for analyzing relationships among cichlid fishes. *J. mol. Evolut.,* **53**: 89-103. https://doi.org/10.1007/s002390010197

Galtier, N., Nabholz, B., Glémin, S. and Hurst, G.D.D., 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Mol. Ecol.,* **18:** 4541-4550. https://doi.org/10.1111/j.1365-294X.2009.04380.x

Hajibabaei, M., Singer, G.A., Hebert, P.D. and Hickey, D.A., 2007. DNA barcoding: How it complements taxonomy, molecular phylogenetics and population genetics. *Trends Genet.,* **23**: 167-172. https://doi.org/10.1016/j.tig.2007.02.001

Herbert, R., Jamtvedt, G., Mead, J. and Hagen, K.B., 2005. Outcome measures measure outcomes, not effects of intervention. *Aust. J. Physiother.,* **51**: 3-4. https://doi.org/10.1016/S0004-9514(05)70047-7

Jacobson, M., Kim, S., Romney, J., Zhu, X. and Frisina, R.D., 2003. Contralateral suppression of distortion-product otoacoustic emissions declines with age: A comparison of findings in CBA mice with human listeners. *The Laryngoscope,* **113**: 1707-1713. https://doi.org/10.1097/00005537-200310000-00009

Kadowaki, S., Ohnishi, K., Arai, S., Guliyev, F. and Nishiaki, Y., 2016. Mitochondrial DNA Analysis of Ancient Domestic Goats in the Southern Caucasus: A Preliminary Result from Neolithic Settlements at Göytepe and Hacı Elamxanlı Tepe. *Int. J. Osteoarchaeol.*, 2534. https://doi.org/10.1002/oa.253

Kibegwa, F.M., Githui, K.E., Jung'a, J.O., Badamana, M.S. and Nyamu, M.N., 2016. Mitochondrial DNA variation of indigenous goats in Narok and Isiolo counties of Kenya. *J. Anim. Breed. Genet.,* **133**: 238–247. https://doi.org/10.1111/jbg.121824

Librado, P. and Rozas, J., 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics,* **25**: 1451-1452. https://doi.org/10.1093/bioinformatics/btp187

Luikart, G., Gielly, L., Excoffier, L., Vigne, J.D., Bouvet, J. and Taberlet, P., 2001. Multiple maternal origins and weak phylogeographic structure in domestic goats. *Proc. natl. Acad. Sci.,* **98**: 5927-5932. https://doi.org/10.1073/pnas.091591198

Parson, W., Pegoraro, K., Niederstätter, H., Föger, M. and Steinlechner, M., 2000. Species identification by means of the cytochrome b gene. *Int. J. Legal Med.,* **114**: 23-28. https://doi.org/10.1007/s004140000134

Petta, G., Rangabasyam, M. and Edger, R., 2004. *U.S. Patent Application No*. 10/829.645.

Pidancier, N., Jordan, S., Luikart, G. and Taberlet, P., 2006. Evolutionary history of the genus Capra (Mammalia, Artiodactyla): discordance between mitochondrial DNA and Y-chromosome phylogenies. *Mol. phylogeny. Evolut.,* **40**: 739-749. https://doi.org/10.1016/j.ympev.2006.04.002

Song, H., Buhay, J.E., Whiting, M.F. and Crandall, K.A., 2008. Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *Proc. natl. Acad. Sci.,* **105:** 13486-13491. https://doi.org/10.1073/pnas.0803076105

Stewart, J.D., Tennant, S., Powell, H., Pyle, A., Blakely, E.L. and Mewasingh, L.D., 2009. Novel POLG1

mutations associated with neuromuscular and liver phenotypes in adults and children. *J. med. Genet.,* **46:** 209-214. https://doi.org/10.1136/jmg.2008.058180

Sultana, S., Mannen, H. and Tsuji, S., 2003. Mitochondrial DNA diversity of Pakistani goats. *Anim. Genet.,* **34**: 417-421. https://doi.org/10.1046/j.0268-9146.2003.01040.x

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evolut.,* **30**: 2725-2729. https://doi.org/10.1093/molbev/mst197

Waugh, D.S., 2005. Making the most of affinity tags. *Trends Biotechnol*., **23**: 316-320. https://doi.org/10.1016/j.tibtech.2005.03.012

Xiufeng, X. and Árnason, Ú., 1994. The complete mitochondrial DNA sequence of the horse, Equus caballus: extensive heteroplasmy of the control region. *Gene,* **148**: 357-362. https://doi.org/10.1016/0378-1119(94)90713-7