IJAS Iranian Journal of Applied Animal Science

Investigating the Origin and Genetic Relationships of Iranian, Akhal-Teke and Arabian Horse Breeds Through Mitochondrial Genome Analysis

Research Article

E. Rezvannejad[1], K. Nobari[2*], M. Asnaashari[3] and S. Ghazanfari[4]

[1] Department of Biotechnology, Institute of Science and High Technology and Environmental Science, University of Advanced Technology, Kerman, Iran
[2] Department of Animal Science Research, Tehran Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Varamin, Iran
[3] Department of Animal Processing, Animal Science Research Institute of Iran (ASRI), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran
[4] Department of Animal and Poultry Science, College of Aburaihan, University of Tehran, Pakdasht, Tehran, Iran

Received on: 19 Jan 2025
Revised on: 4 Feb 2025
Accepted on: 28 Feb 2025
Online Published on: Mar 2025

*Correspondence E-mail: k.nobari@areeo.ac.ir

Online version is available on: www.ijas.ir
https://doi.org/10.71798/ijas.2025.1203001

ABSTRACT

Throughout human civilization, horses have played significant roles in social, cultural, economic, and sporting dimensions. Even today, horses play an important role in human life, particularly in recreational and sporting activities. Understanding the relationships between breeds and genetic diversity can guide the design of necessary strategies to utilize the potential of this noble species. The mitochondrial genome, which is inherited exclusively from the mother to offspring, is widely used for phylogenetic studies. Given the geographical and social proximity of Iran to countries with Arab and Akhal-Teke breeds, the genetic relationship between these breeds and Iranian horses has been investigated in the context of the presence of other breeds worldwide. Therefore, the mitochondrial genome of 174 horses from 78 breeds worldwide was obtained from the NCBI database, and they were analyzed using the CLC Genomic Workbench 23.0.5 software and various packages of the R software. Alignment and phylogenetic tree construction based on the alignment and the Maximum Likelihood method were performed and compared. Finally, the haplotypes resulting from the genomic regions under investigation were examined. In terms of intra-breed diversity, Arab, Iranian, and Akhal-Teke horses ranked highest. The method of phylogenetic tree construction had a significant impact on shape of the resulting tree. Regarding the number of unique haplotypes in the D-loop and CYTB regions, Iranian horses had the highest number of haplotypes and showed closer affinity to the Akhal-Teke breed. In the HVR-1 region, Arab horses had the highest number of haplotypes and showed the greatest affinity with the Akhal-Teke breed. The D-loop haplotype network divided the studied population into two groups, while the HVR-1 haplotype network demonstrated that a more precise and less complex differentiation could be achieved based on it. Finally, the results support the close relationship between Arab, Iranian, and Turkmen horses, indicating a common origin among them.

KEY WORDS genetic diversity, horse breeds, mitochondrial genome, phylogenetic.

INTRODUCTION

Horses have played a significant social and cultural role throughout the history of human civilization, and scientists believe that horses were domesticated from several different populations around 4000 to 6000 years ago (Zhang *et al.* 2012). This domestication occurred during the Neolithic period in various locations worldwide and in western Eurasia (Warmuth *et al.* 2012). Based on the prominent inscriptions remaining from the palaces of ancient Iranian empires,

it is believed that Iran has been a hub for horse breeding since ancient times and held special significance among Iranians. Iranian horses have exhibited a wide genetic diversity and encompass various breeds that have had interactions and influences with other important breeds, such as Turkmen and Arabian breeds. Iranian horses possess different characteristics based on their geographical region. Some of the finest Iranian horse breeds include Arab, Kurd, Qarabagh, Qashqai, Caspian, Turkmen, and Taleshi. Different horse breeds have been selected for various purposes such as sports, transportation, and warfare.

Among them, the selection of horses based on speed, endurance, power, gait, size, color, and body structure has led to the creation of approximately 400 to 500 different horse breeds (Petersen *et al.* 2013). The Arabian horse breed is recognized as the oldest known horse breed, which has been dispersed worldwide to improve the genetics of other breeds (Khanshour and Cothran, 2013). The Akhal-Teke breed, bred by the Turkmen people, is one of the oldest breeds and is native to the southern regions of Turkmenistan (Kang *et al.* 2023). The geographical proximity of the Akhal-Teke horse breeding areas and the presence of Turkmen communities may have influenced the interaction and influence of this breed with Iranian breeds. Alongside examining the phenotypic characteristics, studying the genomic characteristics of horse populations is necessary for identification, preservation of genetic reserves, and determining breeding strategies. Horse breeds can be classified based on phenotypic characteristics, but the true purity of their lineage is often ambiguous due to the lack of pedigree registration in many cases.

Considering the possibility that one of the potential origins of the Arabian horse could be the Iranian plateau, and also aiming to investigate the origins of Iranian horses and their relationship with Arabian and Turkmen horse breeds, it is necessary to design an experimental study that utilizes mitochondrial genome data to examine this matter.

The mitochondrial genome, a small extrachromosomal genome, is approximately 16660 nucleotides long and encodes 37 genes, including 13 protein-coding genes without introns, 22 transfer RNA (tRNA) genes, and 2 ribosomal RNA (rRNA) genes. It is maternally inherited from mother to offspring (Myćka *et al.* 2022). In addition to the mentioned genes, the mitochondrial genome contains a highly variable region known as the control region, specifically the D-loop region, which is further divided into two highly variable regions, HVR1 and HVR2 (Myćka *et al.* 2022). In recent years, the D-loop region and the cytochrome b gene of the mitochondrial genome have received attention for studying genetic diversity within and between breeds due to their greater variability compared to the chromosomal genome and other regions of the mitochondrial genome (Hong *et al.* 2022; Machmoum *et al.* 2023; Nikbakhsh *et al.* 2023). The mitochondrial genome sequence is widely used for the study of classification, phylogenetics, genetic structure, and biological identity. Due to its maternal transmission, the mitochondrial genome plays a significant role in maternal inheritance and is useful for studies on diversity and genetic conservation of the maternal lineage. Individuals share a common maternal lineage in their mitochondrial genome, making it a useful tool for investigating molecular biodiversity, phylogenetics, and species origins. Furthermore, examining the maternal lineage is an important approach for assessing breed purity. Several studies have identified high levels of genetic diversity in Arabian horse breeds (Głażewska and Jezierski, 2004), indicating significant diversity within horses with Arabian ancestry. The findings of the research (Głażewska, 2010) propose a hypothesis that the Arabian breed has emerged from different breeds and populations, and breed purity indicators are associated with current populations of this breed, whose pedigrees do not extend more than 200 years. In an attempt to better understand the origins of Arabian and Turkmen horses, as well as investigate the origins of Iranian horses and their genetic relationship with Arabian and Turkmen horse breeds, this experiment has been designed to utilize mitochondrial genome data to examine this matter.

MATERIALS AND METHODS

Samples

In this study, a total of 174 horses from 78 different breeds worldwide, for which complete mitochondrial genome sequences were available in the NCBI database, were used. The study was conducted using all the data that contained the complete mitochondrial genome sequence. Generally, there were 16,660 nucleotides for each sample, with some samples containing ambiguous nucleotides (N) that were taken into account during sequence alignment. The sequences used in this study included 14 Iranian horses, 11 Arabian horses, and 13 Akhal-Teke horses (Table 1), along with 136 other horses belonging to different breeds.

Genomic fragments

In addition to the whole mitochondrial genome data used for sequence alignment and phylogenetic tree construction, the sequences of cytochrome b, D-loop, and HVR-1 were also separately analyzed. The total length of the mitochondrial genome sequences was 16660 base pairs. The length of the cytochrome b sequence was 1,140 base pairs (positions 14192 to 15321), the length of the D-loop sequence was 1192 base pairs (positions 15469 to 16660), and the length of the HVR-1 sequence, which corresponds to the first part of the D-loop, was 366 base pairs (positions 15469 to 15834).

Genomic data analysis

The whole mitochondrial genome, cytochrome b, D-loop, and HVR-2 genomic regions were analyzed using CLC Genomic Workbench 23.0.5 software. After performing highly accurate sequence alignment, a phylogenetic tree was constructed based on the alignment using the Neighbor-Joining method, which calculates nucleotide distance using the Kimura 80 model. To assess the confidence level of the constructed phylogenetic tree, bootstrapping was performed 100 times. In addition to the phylogenetic tree, pairwise sequence comparisons were also conducted for the performed alignments. In the pairwise comparisons, the number of gaps, differences, distance, percent identity, and identities were calculated. To obtain a maximum likelihood phylogenetic tree using the Expectation Maximization on substitution model parameters and branch length optimization along with topology changes (PHYML), several models including Jukes-Cantor (JC), Felsenstein 81 (F81), Kimura 80 (K80), Hasegawa-Kishino-Yano (HKY), and General Time Reversible (GTR) were compared by incorporating genetic grouping and genetic diversity into the model. These models were compared with each other based on four types of model selection tests, including hierarchical likelihood ratio test (hLRT), BIC, AIC, and AICC. The model that obtained the best performance in most or all of the tests was used. The resulting tree was then compared to the tree obtained from the Kimura 80 alignment method using the Phylo.io software. To obtain the haplotypes and their frequencies, the R software was utilized. The msa package was used for performing multiple alignment analysis. Subsequently, the ape package was employed for phylogenetic and evolutionary analyses, and the resulting phylogenetic tree data were visualized using the ggtree software. The haplotypes were calculated using the haplotypes package. The haplotype distance matrix was computed using the pegas package, employing the Hamming distance method. The haplotype network was then constructed using the Haplotype network function of the same package. For generating a heat map, the haplotype sequences matrix was compared pairwise, and a symmetric matrix was created based on their similarity using the stats package. Finally, the data visualization of the heat map was performed using the visualization functions provided by the stats package.

RESULTS AND DISCUSSION

The alignment of mitochondrial sequences, including CYTB, D-loop, and HVR-1, for the examined samples was obtained using the CLC software. The obtained alignments were utilized to visualize the differences, similarities, and gaps. After visualizing the pairwise differences between the examined sequences, the sequences of the desired populations were examined. The average, maximum, and minimum differences between and within the examined populations in the mitochondrial genome sequence were presented in Table 2. Arabian horses exhibited a higher within-breed diversity in terms of mitochondrial, D-loop, and HVR-1 sequences compared to other groups, with the widest range of differences (the difference between maximum and minimum) observed within this breed. In terms of CYTB sequences, Iranian horses showed a higher within-breed variation compared to the other two breeds. The Akhal-Teke breed had the lowest within-breed differences and the smallest range of differences across all mentioned sequences. In all sequences except for CYTB, Iranian horses had fewer nucleotide differences with the Akhal-Teke breed compared to Arabian horses. The sequence differences between the Arabian breed and the Akhal-Teke breed were lower compared to the differences between the Arabian breed and Iranian horses. Despite its longer length, the CYTB gene exhibited significantly less diversity compared to the D-loop and HVR-1 regions.

The phylogenetic tree was constructed using two methods based on alignment and maximum likelihood.

Phylogenetic trees for each of the examined sequences were generated using the CLC software, as follows (Figure 1). The phylogenetic tree derived from mitochondrial sequence evidence indicates that the Arabian and Akhal-Teke breeds are present in a wider range of the phylogenetic tree and across all haplogroups. Based on the phylogenetic tree generated from mitochondrial sequences, Iranian horses do not have a presence in one of the major haplogroups.

Maximum likelihood-based phylogenetic tree

To construct the phylogenetic tree using the maximum likelihood method for the D-loop, CYTB, and HVR-1 regions, various models were examined. For the D-loop region, hLRT, BIC, and AICC tests determined that the best model was the Kimura 80 model, considering both the grouping and topology. This model ranked second in terms of AIC test with a slight difference (AIC value of 8745 compared to 8757). Therefore, the Kimura 80 method was selected for this region. For the CYTB gene, all test methods evaluated the HKY model along with grouping and topology as the best model. For the HVR-1 region, the Kimura 80 model ranked first using the hLRT test, but received ranks higher than 3 in the other methods. Therefore, the GTR method considering grouping and topology (GTR+G+T), which ranked first in the BIC and AIC tests, was used as the optimal model. Maximum likelihood phylogenetic tree using the best models for D-loop, CYTB and HVR-1 presented in Figure 2. A comparison of different methods for phylogenetic tree visualization was performed using the phylo.io software.

Table 1 Accession numbers of the sequences used for Iranian, Arabian, and Akhal-Teke breeds

Breed	Accession number
Iranian	JN398405, JN398383, JN398395, JN398414, JN398415, JN398419, JN398444, JN398446, JN398445, JN398451, JN398455, JN398457, JN398423, JN398433
Arab	JN398406, JN398412, JN398380, JN398392, JN398448, AP013078, JN398434, HQ439449, HQ439488, HQ439448, HQ439447
Akhal-Teke	JN398410, JN398404, JN398385, JN398393, JN398435, JN398450, JN398449, JN398452, JN398453, JN398422, JN398424, HQ439441, HQ439442

Table 2 Average, maximum, and minimum nucleotide differences between and within the examined populations in mitochondrial genome, D-loop region, CYTB and HVR-1 sequences

Item	Breed[1]	Mean			Maximum			Minimum		
		AkT	Arb	Irn	AkT	Arb	Irn	AkT	Arb	Irn
Mitocgenome	AkT	86.5			122			6		
	Arb	110.7	106.6		218	218		11	11	
	Irn	92.5	101.3	103.6	164	214	162	3	4	14
D-loop	AkT	19			29			3		
	Arb	24.4	30.1		85	97		1	2	
	Irn	20.1	25.8	22.5	33	87	35	0	3	3
CYTB	AkT	0.38			1			0		
	Arb	0.65	0.87		2	2		0	0	
	Irn	0.81	1.06	1.3	3	3	4	0	0	0
HVR-1	AkT	8.8			15			1		
	Arb	8.9	9.7		17	17		0	0	
	Irn	9.1	9.5	10	18	18	18	0	0	2

AkT: Akhal-Teke; Arb: Arab and Irn: Iranian.

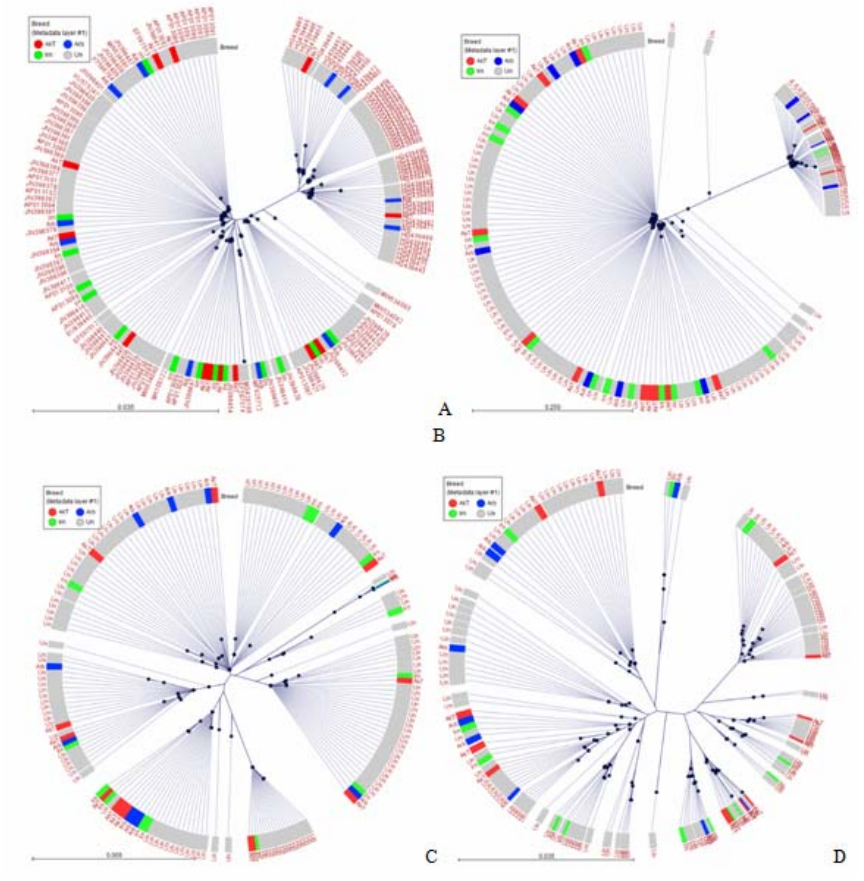

Figure 1 Phylogenetic tree based on alignment using the Kimura 80 method for different samples using mitochondrial (A), D-loop (B), CYTB (C), and HVR-1 (D) sequences

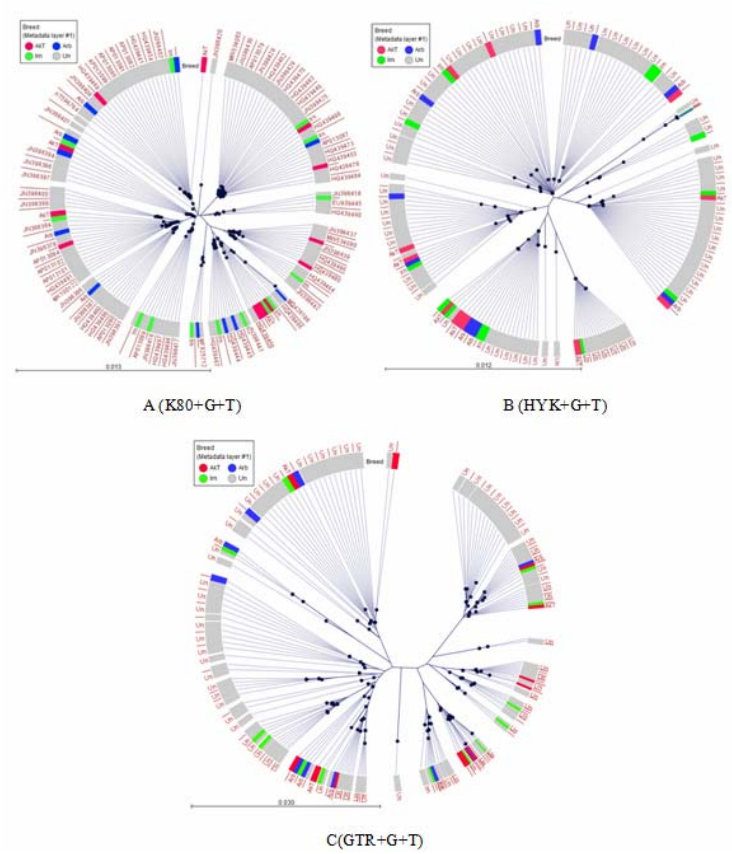

Figure 2 Maximum likelihood phylogenetic tree using the best model for different samples based on D-loop (A), CYTB (B), and HVR-1 (C) sequences

The Figure 3, illustrates the comparison between two methods for each of the sequences. In this comparison, the similarity between the two trees is measured based on the Jaccard index, with the values represented as a color scale in the bottom margin of the figures, corresponding to the tree nodes.

The sequence variants that tend to be inherited together is depicted as haplotypes. The haplotypes of the examined sequences were obtained using R software. Haplotypes are clusters of single nucleotide polymorphism (SNPs) diversity that determine the pattern of inheritance and are associated with the level of conservation in the relevant genomic region.

Investigation of D-Loop region haplotypes revealed that the studied horse population in this region consists of 161 haplotypes. Many of these haplotypes were observed within specific population groups. Among the obtained haplotypes, Iranian horses shared haplotypes 59 and 71 with the Akhal-Teke breed but had no shared haplotypes with the Arabian breed. Iranian horses shared haplotypes 7 and 96 with other horse breeds. Haplotype 17 obtained in this study was shared between the Arabian and Akhal-Teke breeds. The Akhal-Teke breed shared haplotype 90, while the Arabian breed shared haplotype 144 with other breeds.

The Akhal-Teke, Arabian, and Iranian breeds had 6 (6, 15, 38, 84, 89, and 106), 9 (9, 12, 18, 33, 67, 81, 86, 109, and 149), and 10 (19, 22, 31, 51, 55, 62, 68, 69, 82, and 88) specific D-Loop haplotypes, respectively.

In the HVR-1 sequence obtained from horse samples, a total of 87 haplotypes were identified. All horse populations shared haplotype 10, while there were no shared haplotypes between Iranian, Arabian, and Akhal-Teke horses, except for haplotypes 58 and 74, which were shared between Arabian and Akhal-Teke horses. The Iranian, Arabian, and Akhal-Teke horse populations had 5 (haplotypes 7, 53, 60, 82, and 87), 4 (haplotypes 3, 11, 34, and 39), and 6 (haplotypes 9, 31, 42, 44, 73, and 80) unique haplotypes, respectively.

The phylogenetic tree of the different identified haplotypes in the D-loop, CYTB, and HVR-1 sequences was constructed using the treeio and ggtree packages. The genetic distances were calculated using the pegas package, and the tree was plotted using the Neighbor-Joining method with 100 bootstrap replicates (Figure 4).

To gain a proper understanding of the relationships among haplotypes in the phylogenetic tree, a heatmap plot was generated. In this plot (Figure 5), darker cells indicate a closer genetic relationship between haplotypes.

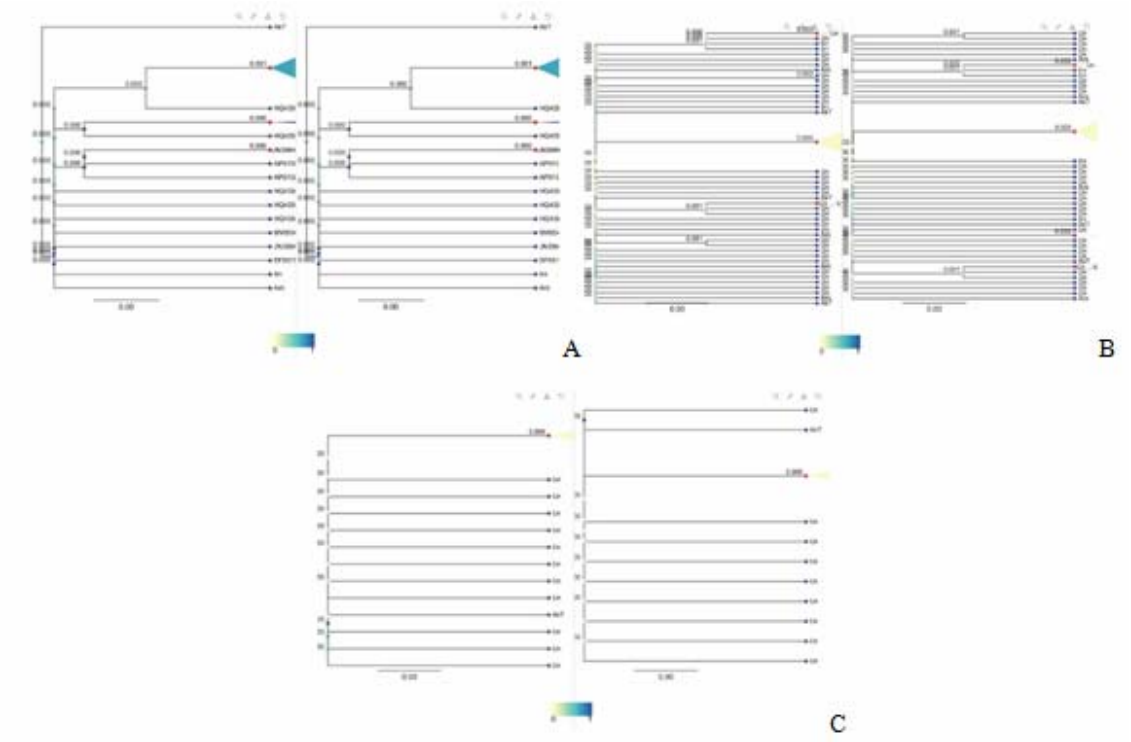

Figure 3 Comparison of the alignment method (left) and maximum likelihood (right) for D-loop (A), CYTB (B), and HVR-1 (C) sequences

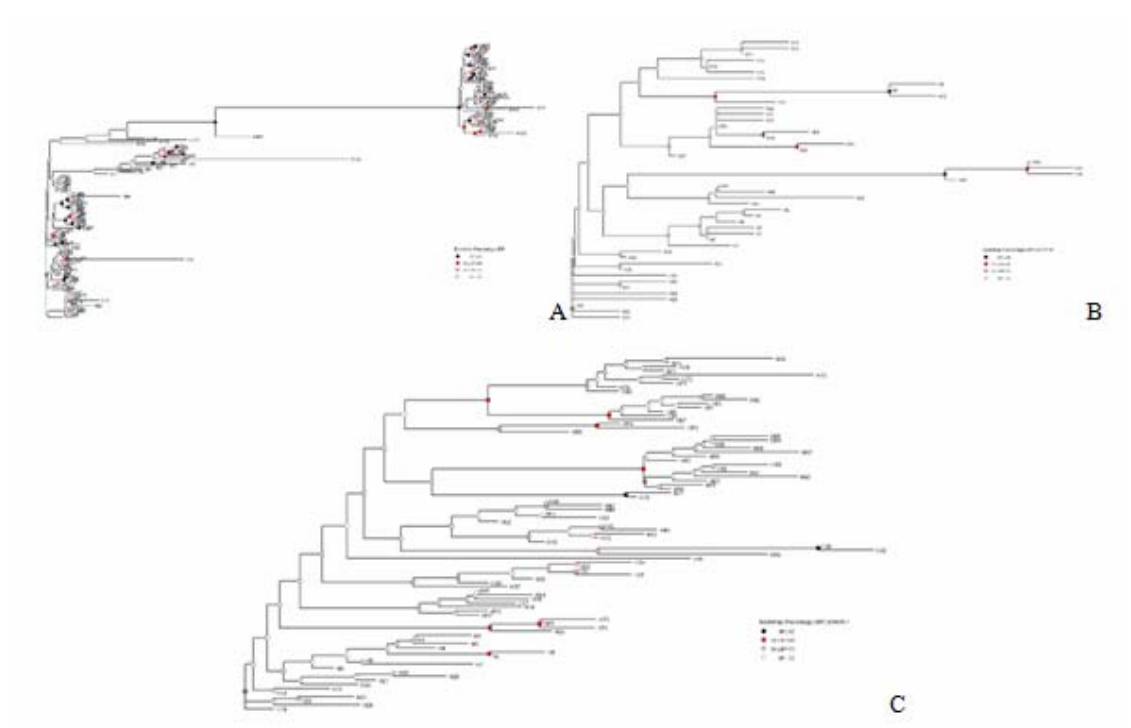

Figure 4 Phylogenetic trees of haplotypes obtained from D-loop (A), CYTB (B), and HVR-1 (C) sequences

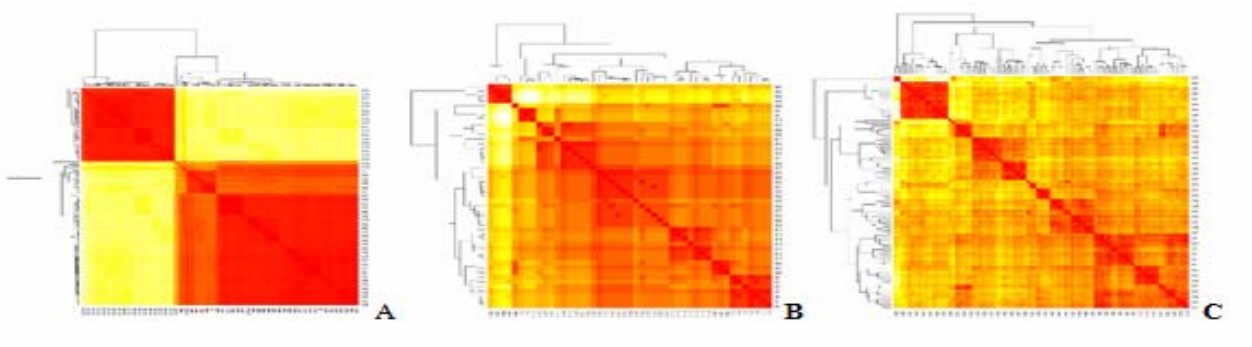

Figure 5 Heatmaps for D-loop (A), CYTB (B), and HVR (C) regions

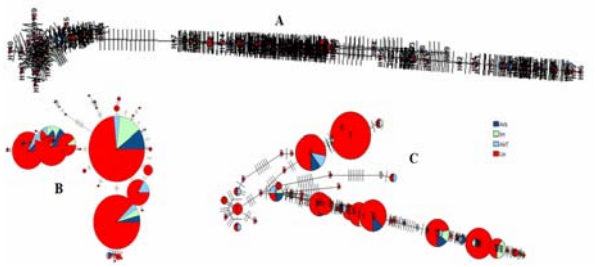

Figure 6 Graphical haplotype network of D-loop (A), CYTB (B), and HVR (C) sequences

The relationships among haplotypes based on the distance matrix between them are presented as a heatmap for the D-loop, CYTB, and HVR-1 regions.

The haplotype network is generated using the pegas package, where the size of the circles represents the number of animals carrying that haplotype, the connecting lines between circles indicate the genetic distance between haplotypes, and each dashed line on the connecting lines represents a nucleotide difference. With these features, the graphical haplotype network for the D-loop, CYTB, and HVR-1 sequences of different horse groups is as follows (Figure 6).

In many studies, the importance of mitochondrial genome has been demonstrated in investigating maternal genetic diversity, population structure, phylogenetics, and even the origin of breeds (Dhorne-Pollet *et al.* 2020; Abdullah, 2023).

Based on within-breed diversity, the Arabian breed exhibited the highest diversity with an average nucleotide difference of 6.106, which is consistent with the findings of Sadeghi *et al.* (2019), who reported a high genetic diversity for Arabian horses based on the genetic similarity criterion. The Akhal-Teke breed showed the lowest within-breed differences in all sequences, indicating a more restricted and limited maintenance of this breed.

The lower nucleotide differences between Iranian horses and the Akhal-Teke breed may indicate a closer relationship. However, considering the discussed differences, the Arabian breed shows a greater affinity with the Akhal-Teke breed than with Iranian horses.

The lower diversity in the CYTB sequence suggests a higher conservation of this gene in horse species compared to the regulatory regions D-loop and HVR-1. According to the distribution of Akhal-Teke and Arabian breeds based on mitochondrial sequence, if we consider this distribution and effective population size as an indicator of diversity, the results of this study are consistent with the research conducted by Mousavi *et al.* (2023), who obtained effective population sizes of 59 and 102 for Iranian Turkmen and Arabian breeds, respectively. The presence of the examined breeds in different haplogroups indicates a high diversity of mitochondrial sequences, as mentioned by Lippold *et al.* (2011).

Based on the results obtained from the comparison of the sequences, there are significant differences (node color) in particular for the HVR-1 sequence between the alignment method and the maximum likelihood method in the two phylogenetic trees. Therefore, considering the findings of Truszkowski and Goldman (2016), which demonstrated the stability of the maximum likelihood method even in the presence of gaps in multiple alignment sequences, it can be concluded that the maximum likelihood method is more reliable.

From the perspective of the D-loop, Iranian horses exhibited greater diversity with 10 unique haplotypes compared to other breeds. In terms of haplotypes obtained from this genomic region, the Iranian breed showed a closer kinship with the Akhal-Teke breed, sharing 2 haplotypes. Despite having a higher nucleotide difference within the population (Table 1) compared to other breeds, the Arabian breed displayed a lower number of haplotypes.

Iranian horses showed a closer kinship with the Akhal-Teke breed in terms of the presence of a shared haplotype specific to the CYTB region. Iranian horses exhibited greater diversity by possessing three exclusive haplotypes compared to the Arabian and Akhal-Teke breeds.

The Arabian breed exhibited the highest diversity in terms of HVR-1 position, with six haplotypes. Furthermore, based on the number of shared exclusive haplotypes, its closer kinship with the Akhal-Teke breed was determined.

Based on the phylogenetic tree of haplotypes obtained from the D-loop region, the studied horse populations are divided into two distinct genetic groups, and the results resemble the phylogenetic tree of sequence alignment of this region. Other genomic regions have also followed a similar pattern, indicating the reliability of the alignment method in constructing the phylogenetic tree.

The heatmap plot for the D-loop region shows that the studied horse population is divided into two distinct groups, indicating no close relationship between these two groups. Only within-group relationships are observed. In the case of the CYTB sequence, the division of haplotypes into smaller groups is evident in the heatmap, but sometimes haplotypes that are positioned further apart in the phylogenetic tree also exhibit close relationships with each other. The heatmap generated from the HVR-1 haplotypes, similar to the CYTB sequence, divides the population into different groups, but the relationships between haplotypes within the population are much more clear-cut.

The haplotype network for the D-loop sequence indicates a high number of haplotypes with multiple nucleotide differences among them. The CYTB haplotype network shows fewer haplotypes, and some haplotypes were found to be more abundant in the studied horse population. In the HVR-1 haplotype network, there were haplotypes with high abundance that were not present in any of the examined horses. Therefore, it can be said that the subdivision based on the sequence diversity of HVR-1 may provide a clearer population differentiation with less complexity compared to the D-loop sequence and CYTB sequence.

Based on the results of the level of differences, the Akhal-Teke and Arabian breeds had the highest and Iranian breed had the lowest average nucleotide difference. Considering that the within-group differences among Iranian horses were greater than their differences with the Akhal-Teke and Arabian breeds, it appears that the Iranian group possesses a high genetic diversity within itself. This finding aligns with the results of the study conducted by Hedayat Evrigh *et al.* (2018), which identified high genetic diversity and abundant maternal lineages in Iranian native horses. Additionally, based on the minimum and maximum difference results, the range of diversity in Iranian breeds is lower compared to other breeds.

Kang *et al.* (2023) proposed the existence of a connection between the Akhal-Teke breed and Middle Eastern horses. They demonstrated that the Akhal-Teke breed did not undergo significant expansion throughout its history. In their study, the Caspian horse and Arabian breed were identified as probable ancestors of the Akhal-Teke horse.

Considering the high diversity of microsatellite markers, another study examining rare alleles in the Akhal-Teke breed found a strong resemblance to the Tuvinian and Khakassian breeds (Leisson *et al.* 2011). In contrast to the theory suggesting the origin of the Akhal-Teke breed from Eurasian steppes, there is also evidence of its origin from the Middle East, including ancient epic poems referring to them as celestial horses and findings from various research studies (Luís *et al.* 2007; Giulotto *et al.* 2017). This study further supports the Middle Eastern origin of the Akhal-Teke breed due to the observed close kinship between the Akhal-Teke, Iranian, and Arabian horses.

Iranian horses exhibit phenotypic variations and are divided into different groups based on their geographical locations. Within each group of Iranian horses, there are distinct phenotypic differences that further divide them into subgroups. For example, Iranian Turkmen horses are categorized into four groups: Yomut, Tekke, Jurgalan, and Chenaran. Except for the last group, which derives its name from the place where Turkmen and Arabian horses crossed to produce a breed that Nader Shah Afshar used for the conquest of India (Avery *et al.* 1991), the names of the other three cases are associated with the tribe of responsible for breeding. Similarly, the Iranian Kurdish horse group is divided into three subgroups: Afshar, Jaf, and Sanjabi, which are distinguishable from each other based on their phenotypic characteristics. This existing diversity in Iranian horses aligns with the findings of this study, which observed a significant diversity within Iranian horses.

CONCLUSION

Overall, this study demonstrates that Arabian horses have the highest genetic diversity, while Akhal-Teke horses have the lowest genetic diversity. In this respect, Iranian horses fall among them. The evidence obtained from this study indicates a close relationship between Turkmen, Arabian, and Iranian horses, supporting a common origin for these breeds.

ACKNOWLEDGEMENT

The authors would like to express my sincere gratitude to the Golestan Agricultural and Natural Resources Research and Education Center for collaboration in this research. Additionally, the authors wish to acknowledge the financial support provided for this project (No. 03/2682) by the Insti-

tute of Science and High Technology and Environmental Sciences at the Graduate University of Advanced Technology, Kerman, Iran.

REFERENCES

Abdullah S. (2023). Mitochondrial DNA sequencing of Kehilan and Hamdani horses from Saudi Arabia. *Saudi J. Biol. Sci*. **30(9),** 1-8.

Avery P., Hambly G. and Melville C. (1991). The Cambridge History of Iran. Cambridge University Press, New York, NY.

Dhorne-Pollet S., Barrey E. and Pollet N. (2020). A new method for long-read sequencing of animal mitochondrial genomes: application to the identification of equine mitochondrial DNA variants. *BMC Genomics*. **21,** 785-797.

Giulotto E., Raimondi E. and Sullivan K.F. (2017). The unique DNA sequences underlying equine centromeres. *Prog. Mol. Subcell. Biol*. **56**, 337-354.

Głażewska I. (2010). Speculations on the origin of the Arabian horse breed. *Livest. Sci*. **129(1),** 49-55.

Głażewska I. and Jezierski T. (2004). Pedigree analysis of Polish Arabian horses based on founder contributions. *Livest. Prod. Sci*. **90(2),** 293-298.

Hedayat Evrigh N., Omri M., Boustan A., Seyedsharifi R. and Vahedi R. (2018). Genetic diversity and structure of iranian horses' population based on mitochondrial markers. *J. Equine Vet. Sci*. **64,** 107-111.

Hong J.H., Oh C.S., Kim S., Kang I.U. and Shin D.H. (2022). Genetic analysis of mitochondrial DNA from ancient Equus caballus bones found at archaeological site of Joseon dynasty period capital area. *Anim. Biosci*. **35(8),** 1141-1150.

Kang Z., Shi J., Liu T., Zhang Y., Zhang Q., Liu Z., Wang J. and Cheng S. (2023). Genome-wide single-nucleotide polymorphism data and mitochondrial hypervariable region 1 nucleotide sequence reveal the origin of the Akhal-Teke horse. *Anim Biosci*. **36(10),** 1499-1507.

Khanshour A.M. and Cothran E.G. (2013). Maternal phylogenetic relationships and genetic variation among Arabian horse populations using whole mitochondrial DNA D-loop sequencing. *BMC Genet*. **14,** 83-93.

Leisson K., Alev K., Kaasik P., Jaakma Ü. and Seene T. (2011). Myosin heavy chain pattern in the Akhal-Teke horses. *Animal*. **5(5),** 658-662.

Lippold S., Matzke N.J., Reissmann M. and Hofreiter M. (2011). Whole mitochondrial genome sequencing of domestic horses reveals incorporation of extensive wild horse diversity during domestication. *BMC Evol. Biol*. **11,** 328-338.

Luís C., Juras R., Oom M.M. and Cothran E.G. (2007). Genetic diversity and relationships of Portuguese and other horse breeds based on protein and microsatellite loci variation. *Anim. Genet*. **38,** 20-27.

Machmoum M., Badaoui B., Petit D., Germot A., El-Alaoui M.A., Boujenane I. and Piro M. (2023). Genetic diversity and maternal phylogenetic relationships among populations and strains of arabian show horses. *Animal*. **13(12),** 2021-2031.

Mousavi S.F., Razmkabir M., Rostamzadeh J., Seyedabadi H.R., Naboulsi R., Petersen J.L. and Lindgren G. (2023). Genetic diversity and signatures of selection in four indigenous horse breeds of Iran. *Hered*. **131,** 96-108.

Myćka G., Klecel W., Stefaniuk-Szmukier M., Jaworska J., Musiał A.D. and Ropka-Molik K. (2022). Mitochondrial Whole D-Loop variability in Polish Draft horses of sztumski subtype. *Animal*. **12,** 1870-1881.

Nikbakhsh M., Varkoohi S. and Seyedabadi H.R. (2023). Mitochondrial DNA D-loop hyper-variable region 1 variability in Kurdish horse breed. *Vet. Med. Sci*. **9(2),** 721-728.

Petersen J.L., Mickelson J.R., Cothran E.G., Andersson L.S., Axelsson J., Bailey E., Bannasch D., Binns M.M., Borges A.S., Brama P., Machado A.D.C., Distl O., Felicetti M., Fox-Clipsham L., Graves K.T., Guérin G., Haase B., Hasegawa T., Hemmann K., Hill E.W., Leeb T., Lindgren G., Lohi H., Lopes M.S., McGivney B.A., Mikko S., Orr N., Penedo M.C.T., Piercy R.J., Raekallio M., Rieder S., Røed K.H., Silvestrelli M., Swinburne J., Tozaki T., Vaudin M., Wade C.M. and McCue M.E. (2013). Genetic diversity in the modern horse illustrated from genome-wide SNP data. *PLoS One*. **8(1),** e54997.

Sadeghi R., Moradi-Shahrbabak M., Miraei Ashtiani S.R., Schlamp F., Cosgrove E.J. and Antcza D.F. (2019). Genetic diversity of Persian Arabian horses and their relationship to other Native Iranian horse breeds. *J. Hered*. **110(2),** 173-182.

Truszkowski J. and Goldman N. (2016). Maximum likelihood phylogenetic inference is consistent on multiple sequence alignments, with or without Gaps. *Syst. Biol*. **65(2),** 328-333.

Warmuth V., Eriksson A., Bower M.A., Barker G., Barrett E., Hanks B.K., Li S., Lomitashvili D., Ochir-Goryaeva M., Sizonov G.V., Soyonov V. and Manica A. (2012). Reconstructing the origin and spread of horse domestication in the Eurasian steppe. *Proc. Natl. Acad. Sci. USA*. **109(21),** 8202-8206.

Zhang T., Lu H., Chen C., Jiang H. and Wu S. (2012). Genetic Diversity of mtDNA d-loop and maternal origin of three Chinese native horse breeds. *Asian-Australasian J. Anim. Sci*. **25(7),** 921-926.