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Evolutionary Relationship Between Arabian Camel Breeds Based on Sequencing of Heat-Stress Genes under Egyptian Conditions

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ABSTRACT

The Arabian camel (*Camelus dromedarius*), which is domesticated in semi-desert environments in Egypt, is one of the neglected animals in the scientific community. Although, it is the best model for studying acclimatization as it is well adapted to withstand severe drought and high temperatures and survive for long periods. Many physiological and behavioral aspects were found to play a role in such adaptations. At the molecular level, it is well known that heat shock genes play an important role in thermotolerance of camels. Therefore, this study was designed to investigate relationship among the most dominant breeds in Egypt (Fellahi, Maghrebi and Sudani) in order to determine the phylogenetic relationship based on sequences of two heat stress genes (HSP family B (small) member 9: HSPB9 and HSP 70 kDa 1B: HSPA1B). Both genes were isolated and partially sequenced from Fellahi, Maghrebi and Sudani Egyptian breeds. It has been deposited in the NCBI database with accession numbers MZ243318, MZ243319, MZ243320, MZ851990 and MZ851991. By studying the phylogenetic tree based on HSPB9 gene sequence, our results showed that Maghrebi and Sudani breeds are closely related whereas, Fellahi breed was found to be more related to the reference breed on database. Relying on HSPA1B gene sequence, it was found that Maghrebi breed is closely related to wild strain (*Camelus ferus*). Additionally, Fellahi breed was separated into distinct group with reference breed, which indicates strength of its relationship to reference origin.

Key words: Arabian camel, Heat stress genes, PCR, Phylogenetic analysis

INTRODUCTION

Camels have been developed over thousands of years because of their ability to produce high-quality meat, milk and fibers in some of the world's most hostile and high temperature environments. According to FAO (2019), there are about 37.5 million heads distributed at different rates all over the world, in Africa 87.1% and in Asia 12.9%. In Egypt, the average number of camels in 2019 was about 119,885, distributed among five breeds: Maghrebi, Somali, Sudani, Fellahi or Baladi and Mowaled. These breeds are used for different purposes (Ramadan and Murayama 2017).

At the molecular level, three breeds (Fellahi, Maghrebi and Sudani) were found to have a high degree of genetic purity with a lower degree of mixing as it is considered a pure breed (Al-Soudy et al. 2018). All Egyptian breeds are single-humped Arabian camels.

At the physiological and behavioral level, the Arabian camel (Camelus dromedarius) is one of the most important livestock species that has the unique ability to adapt to the harsh conditions of Africa. It has adaptation mechanisms that enable it to survive when exposed to prolonged water deprivation, heat stress, scarcity of food resources or poor quality. (Gebreyohanes and Assen 2017). Moreover, it was reported that Camelus dromedarius is an endothermic animal with the ability to fluctuate its body temperature up to 42° C in adaptation to severe heat stress (Thayyullathil et al. 2008; Ouajd and Kamel 2009).

At the genomic level, it was reported that the cellular heat shock response includes broad systemic gene expression across the cellular and organ level and has been categorized into acute (ability to survive heat endurance at the cellular level), ventilation (ability to increase activity at the organ level) and adaptive (short and long) - changes Genetic range) responses (Collier et al. 2008).

Adaptation to high temperature conditions is vital for future animals as heat stress reduces their productivity and significantly affects their health and fertility (Hayes et al. 2013). At the molecular level, temperature greatly influences on the biological and physiological functions of heterozygous

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organisms (Ackerman et al. 2000). Interestingly, the response to heat shock in mammals plays a vital role in heat endurance as it is a highly conserved process influencing molecular and biochemical processes. Heat shock proteins have been widely used as biomarkers of biotic and abiotic stresses (Bierkens 2000). The most studied isoform among HSPs is HSP70 because it is closely related to chemical and environmental stresses (Hochachka et al. 2002).

All living organisms respond to harsh environmental factors by increasing gene expression and protecting the cell from the harmful effects of protein degradation within cells. These genes are expressed for heat shock proteins (HSPs) (Pruski and Dixon 2007; Monari et al. 2011). Some of these genes as Heat Shock Protein Family B (small) member 9 gene, which coding for HSP, family B (small) Member 9 protein. ID - 105090452. Accession number transcript XM 010981776.2, and its length 515 bp (Elbers et al. 2019) and Heat Shock Protein 70 kDa family 1B gene, also known as HSPA1B.ID 105087446. Accession N transcript XM 010978109.2, its length 2204 bp (Elbers et al. 2019). Thus, the aim of this study is 2 folds; 1. It supplies databases with new sequences of Egyptian camel breeds by isolating and characterizing some of these genes encoding HSPs in three Egyptian camel breeds (Fellahi, Maghrebi and Sudani); 2. To study the relationship between these breeds based on the sequence of these genes.

MATERIALS AND METHODS

Samples Collection

The animal studies were carried out according to the guidelines of the Animal production research Institute, Agriculture research Center (ARC) Giza, Egypt, for Animal Care and Use in the experiments. All procedures in studies involving animals were performed in compliance with The ARRIVE guidelines 2.0 (Animal Research: Reporting In Vivo Experiments).

Blood samples were collected from the Jugular vein of all studied camel breeds (Fellahi, Maghrebi and Sudani). Fellahi and Sudani are located in the Middle of Delta (Menoufia and Qalyubia governorates); the Maghrebi breed was obtained from the Camel Research Center at Matrooh governate. The blood samples were collected at temperatures between 28°C and 34°C in a tube containing 0.5mL EDTA (0.5M) as an anticoagulant, studied animals aged between 7 and 10 years old and 500-600kg of live body weight.

DNA Extraction

From the whole blood samples, total genomic DNA was extracted by using Gene JET Genomic DNA Purification Kit (Thermo Scientific, Cat. No. K0721) according to manufacturer's procedure.

Primer Synthesis

Fifty nmol of all primers were synthesized in China by Quintarabio lab (Customer Support Focused DNA Sequencing), as shown in Table 1. The synthesized primer was diluted by sterilized distilled water to 100 ρ mol and kept under -20°C.

Amplification of HSPB9 and HSPA1B Genes

PCR amplified HSPB9 and HSPA1B genes for all samples. The PCR reaction mixture (50μ L volume) contained 25μ L Thermo Scientific DreamTaqTM Green PCR

Table 1: List of primers designed to identify the heat stress genes

Gene	Primer	Sequence
HSPB9	Forward	GTCGGTAGCAGTCTCCCCAA
	Reverse	AGTGGTTTACTGGGTCAGGCT
HSPA1B	Forward	TTCAAGAGGAAGCACAAGAAG
	Reverse	GGCAGTATACATTCACCTCTAC

Master Mix (2X), 2μ L Forward and Reverse primer (10pmol/ μ L), 2μ L DNA template (50ng/ μ L), 19μ L Water nuclease-free. The PCR program for HSPB9 and HSPA1B genes was at Initial denaturation at 95°C for 6 minutes, then 35 cycles of denaturation at 95°C for 30s, 1min for HSPB9 annealing at 57°C, 60°C for HSPA1B annealing, 1min extension at 72°C, and 7min final extension at 72°C and then kept at 4°C until farther use. Gradient Annealing Temperatures (52 to 60°C) were programmed for all primers to get optimum annealing temperature.

Gel Electrophoresis and Amplified Fragments Visualization and Sequencing

After PCR reactions were performed, from each PCR product, 10μ L were electrophoresed in 2% agarose gel against 100bp DNA ladder as a marker using 1X TAE as a running buffer; gels were stained with Ethidium bromide. By using the standard Sanger method, PCR products were sequenced on ABI 3730XL DNA Sequencer at Macrogen sequencing services (Macrogen, Seol, South Korea).

Sequences Analysis and Accessions Number

The quality of the data generated from the sequencer was checked by the BioEdit program version 7.2.6. the sequenced genes were submitted to NCBI GenBank using Bankit tool (http://www.ncbi.nlm.nih.gov/BankIt/). Additionally, the sequenced genes were searched against the nucleotide collection database (nr/nt) using NCBI BLASTN online tool, considering the default parameters in order to identify their homologous counterparts.

Phylogenetic Tree Construction

The evolutionary relationship was constructed using the Neighbour-Joining method integrated with MEGA software (Kumar et al. 2016). To represent the evolutionary history of the analyzed taxa, an introductory consensus tree is taken from 1000 replicates (Felsenstein 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were analyzed using the p-distance method (Nei and Kumar 2000) and are in the number of base differences per site. All positions containing gaps and missing data were eliminated. In the final dataset were 724 positions.

RESULTS

Amplification of HSPB9 and HSPA1B genes and Visualization

PCR products of three Egyptian breeds yielded two distinct bands for each. As shown in Fig. 1, three clear bands were obtained at 446 bp with annealing temperature 57° C in lanes 2, 3, 4 as a result of PCR using HSPB9



Fig. 1: PCR amplified fragments using HSPB9 and HSPA1B primers, M = 100 bp DNA ladder. Lane2,3,4.446 bp amplified fragment primer (HSPB9) for Fellahi, Sudani and Maghrebi breeds respectively. Lane5,6 and 7. 750 bp amplified fragment primer (HSPA1B) for Sudani, Maghrebi and Fellahi breeds, respectively.



Fig. 2: Phylogenetic tree showing the genetic distances among the three Egyptian camel breeds (Fellahi, Maghrebi and Sudani) and other organisms based on *HSPB9* gene sequence.



Fig. 3: Phylogenetic tree shows the genetic distances among the three Egyptian camel breeds (Maghrebi and Fellahi) and other organisms based on *HSPA1B* gene sequence.



Fig. 4: Graphical alignment shows the locus of HSPB9 fragment submitted sequences for all breeds.

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Fig. 5: The graphical alignment shows the locus of HSPA1B fragment submitted sequences for Maghrebi and Fellahi breeds.

Table 2: Accession number for all the sequences which summited on database

Table 2: Accession number for an the sequences which summited on database											
Gene	Source	product	Length	Breed	Reference breed	Accession No					
HSPB9	Egyptian Camelus	heat shock protein family B	446	Fellahi		MZ243318					
	dromedaries	(small) member 9	446	Maghrebi		MZ243319					
	Breeds		446	Sudani		MZ243320					
HSPA1B		heat shock 70 kDa protein	734	Fellahi	North African dromedary	MZ851990					
		1B	758	Maghrebi		MZ851991					

primers (GTCGGTAGCAGTCTCCCCAA and AGTGGT TTACTGGGTCAGGCT). Additionally, other three clear bands were obtained at 750 bp with annealing temperature 60°C in lanes 5, 6, 7 as a result of PCR using HSPA1B primers (TTCAAGAGGAAGCACAAGAAG and GGCA GTATACATTCACCTCTAC). of note, the mentioned annealing temperatures for PCR outperformed all tested annealing temperatures, these results verified the existence of HSPB9 and HSPA1B genes in the three Egyptian breeds under investigation.

Sequencing and Submission of PCR Fragments

Bidirectional sequencing for PCR products generated was processed for screening the quality using Finsh TV software (Geospiza, Seattle, WA, USA). The chromatogram of each base showed high quality of HSPB9 and HSPA1B sequence peaks. The HSPB9 and HSPA1B fragment sequences have been used as a replicate to the evaluation of sequence accuracy. In addition, the correct nucleotide sequence of two fragments amplified by HSPB9 and HSPA1B primers for all breeds was obtained from the alignment between forward and reverse sequence using CLC Sequence viewer program (Qiagen, Germany). Then the sequenced fragments corresponding to HSPB9 and HSPA1B genes in 3 different Egyptian breeds were submitted in the NCBI database under five different Accession numbers as shown in Table 2.

Molecular Evolutionary Genetic Analysis

To gain new insights and validate the findings obtained from BLASTn search, homologous sequences of HSPB9 and HSPA1B gene were selected for multiple sequence alignment (MSA) using ClustalW implemented in the MEGA 7.0 software package using Neighbor-Joining (NJ) method. The Neighbor-Joining genetic distance (NJ) was calculated using the p-distance method to validate the genetic links among three Egyptian camel breeds based on sequencing of HSPB9 and HSPA1B gene and a phylogenetic tree for HSPB9 (Fig. 2) and HSPA1B (Fig. 3) was created, A phylogenetic tree that explains the closest pairwise genetic distance between the Fellahi and North African dromedary (Camelus dromedarius) breeds, which belong to the same clade (Fig. 2). Similarly, the lowest pairwise genetic distance was documented between Maghrebi and Sudani breeds, located in the same group. The most genetic distance was found between Fellahi and Maghrebi breeds. It suggests that the Maghrebi and Sudani breeds are more closely linked and share common origins, whereas the Fellahi breed is closer to the African breed (the model animal). Fellahi breed is close relative to Sudani breed. Phylogenetic tree for the other gene (HSPA1B) revealed that the Fellahi breed is more closely connected to Camelus dromedarius (African breed) as an animal model than the Maghrebi breed, which separated from Camelus Ferus in one clade, indicating that they share a common ancestor (Fig. 3).

DISCUSSION

Genetic characterization and assessment of genetic diversity are essential steps in the conservation and management of genetic resources. To determine genetic characterization and diversity within and between populations, many procedures, such as biochemical and molecular techniques, can be applied (Abri and Faye 2019; Piro 2021). The high quality of dromedary camel genome sequences, as well as comparative genome analysis, provides a better understanding of camel adaptations to the harsh desert environment (Warda et al. 2014). Many genes involved in immunological and stress responses evolved faster in camels than cattle. Identifying important genes involved in desert adaptation could have implications for breeding programs and bring new insights and possibilities for disease resistance studies in many animal species. As a result, our research, our research aims to provide databases

with a wealth of information about Egyptian camel breeds, so we isolated some heat stress genes and sequenced them to obtain the most important findings, which confirmed the existence of the HSPB9 and HSPA1B genes in the three Egyptian breeds under investigation. In a BLASTN investigation of three Egyptian camel breeds (Fellahi, Maghrebi, and Sudani) against a nucleotide database, functional annotation of HSPB9 revealed height homology to the North African dromedary camel with the length of the gene was 515 bp (NC 044526), (Elbers et al. 2019), 515bp of *Camelus ferus* (Wild Bactrian camel) (NC 045711), and 515 bp of Camelus bactrianus (Bactrian camel) (NW 011517136) (Schoch et al. 2020). With 100% identity for the Fellahi breed and 99.78% for the other breeds. For three breeds, the query coverage was 86%. The length of the gene in Vicugna pacos (XM-006199491.3) was 511bp (Richardson et al. 2019), with percentages of Identity 98.43% and query coverage 87% for all breeds. These records were obtained from a genomic sequence and were predicted by automated computer analysis. As shown in Fig. 4, graphical alignment indicates the locus of HSPB9 fragment supplied sequences for all breeds.

On the other side, searching the submitted HSPA1B (Accession sequence fragments numbers. gene MZ851990.1, MZ851991.1) against nucleotide database using BLASTN online tool returned 99.78%, 99.50% similarity with the reference sequence (Elbers et al. 2019), respectively. Fragment with accession MZ851990.1, MZ851991.1 represent percent identity 99% and query coverage 32 and 35% for both breeds. The two fragments represent identity 30 and 31% query coverage of *camelus* ferus (Schoch et al. 2020). The graphical diagram alignment between these fragments revealed that the submitted fragments consisted of partial CDS as shown in Fig. 5.

Molecular Evolutionary Genetic Analysis

Molecular research on camel HSPs would contribute to our understanding of HSPs and consolidate the unique physiological features observed in Arabian camels (Hoter et al. 2019). Moreover, phylogenetic analysis can reveal a great deal about a population's past demographics as well as the level of genetic variation within and between species. Phylogenetic relationships are generally calculated using computer programs with numerous mathematical models that have been created in recent years (Toparslan et al. 2020).

Many studies on estimating the evolutionary relationship and genetic diversity, as well as the phylogenetic analysis between breeds, use molecular markers such as microsatellites (Manee et al. 2020), or microsatellite and start codon targeted (SCoT) markers (Al-soudy et al. 2018), or Mitochondrial ATP6 and ATP8 genes sequence (Yi Li et al. 2017), or mitochondrial sequence variations (Ming et al. 2017; Ming et al. 2021).

We built a phylogenetic tree based on the HSPB9 HSPA1B genes to better understand genetic diversity across and within Egyptian camel breeds. Fig. 4, shows a phylogenetic tree that explains the closest pairwise genetic distance between the Fellahi and Northern African camel as reference breeds, which belong to the same clade. Similarly, the lowest pairwise genetic distance was documented between the Maghrebi and Sudani breeds, which are located in the same group. The most genetic distance was found between the Fellahi and Maghrebi breeds. It suggests that the Maghrebi and Sudani breeds are more closely linked and share common origins, whereas the Fellahi breed is closer to the African breed (the model animal). Fellahi breed is close relative to Sudani breed. These findings are nearly similar to those of (Al-Soudy et al. 2018), who found that evolutionary relationships based on SCoT and microsatellite markers showed that Maghrebi was separated into a single cluster and the second cluster included two sub-clusters. Sudani formed one sub-cluster, and Fellahi was in the second sub-cluster. Furthermore, the highest genetic distance was between Fellahi and Maghrebi breeds. This result showed semi disagreement with what they reported that the evolutionary relationship between five Egyptian camel breeds showed two groups based on RAPD and microsatellite markers which declared that the first group includes Fellahi and Maghrebi, and the other group contains Sudani.

Depending on the sequence of the HSPA1B gene in the study of the genetic distance between the Egyptian breeds, it was revealed that the Fellahi breed is more closely connected to Camelus dromedarius (African breed) as an animal model than the Maghrebi breed, which separated with Camelus ferus in one clade, indicating that they share a common ancestor. These findings are in agreement with El-Seoudy et al. (2008), who stated in his study on molecular genetic identification of some Egyptian camel breeds based on ISSR that the Maghrebi was independent of the Fellahi breed in single clad, there is a disagreement with (Mahrous et al. 2011) as they reported that the evolutionary relationships between five Egyptian camel breeds showed two groups based on RAPD and microsatellite and markers. The first group includes Baladi and Maghrebi, while the second includes Sudani. Moreover, that the fragment of Sudani breed would not be identified with this gene due to issues with final product of the sequencer.

Conclusion

In conclusion, our data might improve the information on the genetic background of thermotolerance of the Egyptian camel breeds. Additionally, our results revealed the genetic diversity of HSPB9 and HSPA1B genes among different camel breeds. And this genetic diversity may be associated with some differences in their thermotolerance ability.

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Author's Contribution

Prof. Yousry Dowidar developed the idea of research and experiment design Prof: Alaa Zeidan initially wrote the manuscript Prof: Magdy Ramadan Badr reviewed the manuscript and wrote it in its final form Dr: Waleed Shaaban performed bioinformatics analysis Ph.D. student: Ahmed Shehab conducted the experiment and collected samples.

REFERENCES

- Piro M, 2021. Aspects of molecular genetics in dromedary camel. Frontiers in Genetic 12: 1976. <u>https://doi.org/10.3389/</u> fgene.2021.723181
- Abri MA and Faye B, 2019. Genetic improvement in dromedary camels: challenges and opportunities. Frontiers in Genetic 10: 167. https://doi.org/10.3389/fgene.2019.00167
- Ackerman PA, Forsyth RB, Mazur CF and Iwama GK, 2000. Stress hormones and the cellular stress response in salmonids. Fish Physiology and Biochemistry 23: 327–336. https://doi.org/10.1023/A:1011107610971
- Al-Soudy A, El-Sayed A, El-Itriby HA and Hussein EH, 2018. Assessment of the genetic diversity, breeds structure and genetic relationships in four Egyptian camel breeds using microsatellite and start codon targeted (SCoT) markers. Journal of Biodiversity and Endangered Species 6: 2-8. <u>https://doi.org/10.4172/2332-2543.S2-001</u>
- Bierkens JG, 2000. Applications and pitfalls of stress proteins in biomonitoring. Toxicology 153: 61–72. <u>https://doi.org/</u> <u>10.1016/s0300-483x(00)00304-8</u>
- Collier RJ, Collier JL, Rhoads RP and Baumgard LH, 2008. Invited review: Genes involved in the bovine heat stress response. Journal of Dairy Science 91: 445-454. <u>https://doi.org/10.3168/jds.2007-0540</u>
- Elbers JP, Rogers MF, Perelman PL, Proskuryakova AA, Serdyukova NA, Johnson WE, Horin Petr, Corander Jukka, Murphy David and Burger PA, 2019. Improving Illumina assemblies with Hi-C and long reads: an example with the North African dromedary. Molecular Ecology Resources 19: 1015-1026. https://doi.org/10.1111/1755-0998.13020
- El-Seoudy AA, El-Salam AZ, Tharwat EE and EL-Salam FA, 2008. Molecular genetic identification of some camel breeds in Egypt. Egyptian Journal of Genetic and Cytology 37: 191-208
- FAO, 2019. Rome. Italy. FAOSTAT.
- Felsenstein J, 1985. Phylogenies and the comparative method. The American Naturalist 125: 1-5. <u>https://doi.org/10.1086/284325</u>
- Gebreyohanes GM and Assen MA, 2017. Adaptation Mechanisms of Camels (Camelus dromedarius) for Desert Environment: A Review. Journal veterinary Science Technology 8: 1-5. <u>https://doi.org/10.4172/2157-7579.</u> <u>1000486</u>
- Hayes BJ, Lewin HA and Goddard ME, 2013. The future of livestock breeding: genomic selection for efficiency, reduced emissions intensity, and adaptation. Trends in Genetic 29: 206-214. <u>https://doi.org/10.1016 /j.tig. 2012</u> .11.009
- Hochachka PW and Somero GN, 2002. Biochemical Adaptation: Mechanism and Process in Physiological Evolution. Oxford University Press, Oxford, pp: 290.
- Hoter A, Rizk S and Naim HY, 2019. Cellular and molecular adaptation of arabian camel to heat stress. Frontiers in Genetic 10: 588. <u>https://doi.org/10.3389/fgene.2019.00588</u>
- Kumar S, Stecher G and Tamura K, 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870-1874. <u>https://doi.org/10.1093/molbev/msw054</u>
- Mahrous KF, Ramadan HA, Abdel-Aziem SH, Abd-El Mordy M and Hamdan DM, 2011. Genetic variations between camel breeds using microsatellite markers and RAPD techniques. Journal Applied Bioscience 39: 2626-2634.
- Manee MM, Algarni AT, Alharbi SN, Al-Shomrani BM, Ibrahim MA, Binghadir SA and Al-Fageeh MB, 2020. Genome-wide

characterization and analysis of microsatellite sequences in camelid species. Mammal Research 65: 359-373. https://doi.org/10.1007/s13364-019-00458-x

- Ming L, Siren D, Yi L, Hai L, He J and Ji R, 2021. Mitochondrial DNA variation and phylogeography of Old-World camels. Animal Bioscience 34: 525-532. <u>https://doi.org/10.5713/</u> ajas.20.0319
- Ming L, Yi L, Sa R, Wang ZX, Wang Z and Ji R, 2017. Genetic diversity and phylogeographic structure of Bactrian camels shown by mitochondrial sequence variations. Animal Genetics 48: 217-220. <u>https://doi.org/10.1111/age.12511</u>
- Monari M, Foschi J, Rosmini R, Marin MG and Serrazanetti GP, 2011. Heat shock protein 70 response to physical and chemical stress in *Chamelea gallina*. Journal of Experimental Marine Biology and Ecology 397: 71-78. https://doi.org/10.1016/j.jembe.2010.11.016
- Nei M and Kumar S, 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.
- Ouajd S and Kamel B, 2009. Physiological particularities of dromedary (*Camelus dromedarius*) and experimental implications. Journal of Laboratary Animal Scince 36: 19-29. https://doi.org/10.23675/sjlas.v36i1.165
- Pruski AM and Dixon DR, 2007. Heat shock protein expression pattern (HSP70) in the hydrothermal vent mussel Bathymodiolus azoricus. Marine Environmental Research 64: 209-224. <u>https://doi.org/10.1016/j.marenvres.2007.</u> 01.003
- Ramadan S and Murayama M, 2017. Advances in camel genomics and their applications: A review. The Journal of Animal Genetics 45: 49–58. <u>https://doi.org/10.5924/abgri. 45.49</u>
- Richardson MF, Munyard K, Croft LJ, Allnutt TR, Jackling F, Alshanbari F, Jevit M, Wright GA, Cransberg R, Tibary A, Perelman P, Appleton B and Raudsepp T, 2019. Chromosome-Level Alpaca Reference Genome VicPac3.1 Improves Genomic Insight Into the Biology of New World Camelids. Journal of Frontiers in Genetic 10: 586. <u>https://doi.org/10.3389/fgene.2019.00586</u>
- Schoch CL, Ciufo S, Domrachev M, Hotton CL, Kannan S, Khovanskaya R, Leipe D, Mcveigh R, O'Neill K, Robbertse B, Sharma S, Soussov V, Sullivan JP, Sun L, Turner S and Karsch I, 2020. NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database (Oxford). <u>https://doi.org/10.1093/database/baaa062</u>
- Thayyullathil F, Chathoth S, Hago A, Wernery U, Patel M and Galadari S, 2008. Investigation of heat stress response in the camel fibroblast cell line dubca. Annals of the New York Academy of Sciences 1138: 376-384. <u>http://doi.org/10.1196/ annals.1414.039</u>
- Toparslan E, Karabag K and Bilge U, 2020. A workflow with R: Phylogenetic analyses and visualizations using mitochondrial cytochrome b gene sequences. Plos One 15: e0243927. https://doi.org/10.1371/journal.pone.0243927
- Warda M, Prince A, Kim HK, Khafaga N, Scholkamy T, Linhardt RJ and Jin H, 2014. Proteomics of old-world camelid (Camelus dromedarius): Better understanding the interplay between homeostasis and desert environment. Journal of Advanced Research 5: 219-242. <u>https://doi.org/10.1016/j.jare.2013.03.004</u>
- Yi Li, Yisi Ai, Liang Ming, Le Hai, Jing He, Fu-Cheng Guo, Xiang YQ and Rimutu Ji, 2017. Molecular diversity and phylogenetic analysis of domestic and wild Bactrian camel populations based on the mitochondrial ATP8 and ATP6 genes. Livestock Science 199: 95-100. <u>http://dx.doi.org/</u> 10.1016/j.livsci.2017.03.015