Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

In Vivo Genetic Profiling and In Silico Functional Insights into Mitochondrial COX-3 Gene Diversity of Egyptian *Camelus dromedarius*

Sekena H. Abdel-Aziem*, Dalia M. Mabrouk, Heba A. M. Abd El-Kader, Sally S. Alam and Othman E. Othman

Cell Biology Department, Biotechnology Research Institute, National Research Centre, Giza, Egypt.

Abstract

STUDYING the genetic diversity of Egyptian camel populations is critical for evaluating their adaptability to extreme desert environments and guiding conservation strategies. This study analyzed genetic variation in three *Camelus dromedarius* populations—Baladi, Sudani, and Maghrebi—using the mitochondrial COX-3 gene. Genomic DNA was extracted from 90 blood samples (35 Baladi, 30 Sudani, 25 Maghrebi), and a 524-bp COX-3 segment was amplified and sequenced. Two haplotypes were identified, characterized by synonymous SNPs at positions 280 (C>T) and 325 (A>G), preserving Histidine (position 94) and Leucine (position 110). These haplotypes (GenBank accessions: OP994029, OP994030) showed high similarity to *Camelus dromedarius* but moderate divergence from *Camelus bactrianus*. Functional conservation of amino acid sequences (accessions: WHO17330.1, WHO17331.1) was confirmed. Environmental correlations revealed Haplotype 1 predominated in Baladi and Sudani populations from hotter, arid inland regions, while Haplotype 2 was frequent in Maghrebi camels inhabiting milder coastal zones. The observed COX-3 variation suggests mitochondrial gene adaptations to environmental stressors, underscoring their role in climate resilience. These findings emphasize the need to conserve camel genetic diversity to enhance sustainability amid changing climatic conditions.

Keywords: *Camelus dromedarius*, COX-3 gene, environmental adaptation, genetic variation, SNPs

Introduction

The dromedary camel (*Camelus dromedarius*) is an extraordinary species that has adapted to some of the harshest environments on Earth. Originating from the Arabian Peninsula and North Africa, camels are uniquely suited to life in arid and semi-arid regions where they thrive in extreme temperatures, dehydration, and scarcity of resources. Over millennia of domestication, dromedary camels have developed a range of physiological and behavioural adaptations that allow them to survive in deserts, including their ability to regulate body temperature, resist dehydration, and metabolize food efficiently [1]. These exceptional traits make camels essential to human populations in desert ecosystems, providing transportation, food, and other vital resources. Understanding the genetic basis of these adaptations is crucial for improving camel breeding strategies and ensuring their long-term survival as a livestock species in the face of environmental challenges, such as climate change [2].

Mitochondrial DNA (mtDNA) has been widely used in population genetics and evolutionary studies due to its maternal inheritance, rapid mutation rate, and lack of recombination [3]. Mitochondria play a central role in cellular energy production, making their genetic material a key focus in understanding metabolic adaptations to environmental stress. The cytochrome c oxidase subunit 3 (COX-3) gene, which encodes a subunit of the mitochondrial cytochrome c oxidase complex, is crucial for oxidative phosphorylation the process by which cells produce ATP. Because of its role in energy metabolism, COX-3 is a valuable marker for studying how camels adapt genetically to extreme environments where energy efficiency is critical for survival [4, 5].

Cyclooxygenase (COX) genes play a critical role in the regulation of inflammatory responses, and their evolutionary dynamics have been studied in various mammalian species for insights into adaptive traits. In camels (*Camelus dromedarius*), known for their exceptional ability to survive in

*Corresponding authors: Sekena H. Abdel-Aziem, E-mail: nadanahl28@gmail.com Tel.: 01227337810
(Received 01 May 2025, accepted 12 June 2025)
DOI: 10.21608/ejvs.2025.380631.2816

arid environments, the study of COX gene haplotypes is particularly relevant for understanding molecular mechanisms of environmental adaptation. Variations in COX gene haplotypes, such as HAP1 and HAP11, may reflect functional differences that contribute to the camel's physiological resilience, including thermotolerance, water conservation, and stress response mechanisms [6;7].

The identification and comparison of haplotypes like HAP1 and HAP11 can provide insights into selective pressures that shaped the camel genome, potentially highlighting adaptive mutations associated with desert survival. Such polymorphisms in regulatory or coding regions of the COX gene could influence gene expression or enzyme activity, offering a genetic basis for adaptive phenotypes [8;9].

Genetic diversity in camel populations has been studied primarily through mitochondrial DNA, with findings showing relatively low diversity, which is typical for species that have been domesticated for long periods and subjected to restricted gene flow [10;11]. However, even within these low-diversity populations, variations in mitochondrial genes such as COX-3 can offer insights into how camels have adapted to specific environmental conditions. Previous studies suggest that mitochondrial gene variations can be linked to climatic factors such as temperature fluctuations, humidity, and food availability, which directly influence energy demands and stress responses [1].

Additionally, we used Phyre2, a widely used protein structure prediction tool, to model the 3D structure of the COX-3 protein encoded by the identified haplotypes. Phyre2's homology-based modeling approach allows for an in-depth analysis of protein structure, providing insights into how synonymous mutations those that do not alter amino acids could still affect protein folding, stability, and function. Even though synonymous mutations are often considered neutral, research has shown that they can influence protein expression and efficiency, particularly in essential proteins like COX-3 that are critical for mitochondrial function and energy metabolism [12;13]. This structural analysis allows us to examine whether the identified synonymous mutations might play a role in enhancing the protein's function under environmental stress conditions.

The aim of this work is to explore the genetic diversity of Egyptian *Camelus dromedarius* populations by analyzing the COX-3 gene, to identify environmental correlations between genetic variation and local climatic conditions, and to investigate the potential structural and functional implications of synonymous mutations in the COX-3 protein. By combining genetic data with protein structure modeling, this study seeks to provide a comprehensive understanding of how mitochondrial genetic variation contributes to the adaptation of camels to extreme environments.

Material and Methods

Sample Collection and DNA Extraction

Blood samples were collected from three camel populations in Egypt: 35 Baladi camels from El-Shweikhi Farm, Qalyubia; 30 Sudani camels from the Khattab Camel Farm, Nubariya; and 25 Maghrebi camels from the National Research Center farm at King Mariout Research Station in Alexandria. The privately owned camels were sampled with the informed consent of their respective farm owners. All sample collections followed ethical guidelines approved by the Ethics of Medical Research Committee at the National Research Centre, Cairo, Egypt (Approval Number: 12440723). For sample collection, camels were restrained by experienced handlers to minimize stress, and blood was drawn via jugular venipuncture using sterile needles and vacutainer tubes. No euthanasia or sacrifice of animals was involved in this study.

Genomic DNA was extracted from the collected blood samples using the phenol/chloroform method, as described by Wajid et al. [14]. DNA quality and concentration assessed using a NanoDrop spectrophotometer (Thermo Scientific, USA). Extracted DNA was stored at -20°C until further use.

PCR Amplification of the COX-3 Gene

A 524-bp fragment of the mitochondrial COX-3 gene was amplified using polymerase chain reaction (PCR). Each 50 µL PCR reaction mixture contained 100 ng of genomic DNA, 10 pmol of universal primers (forward: 5'-CCAGTGATGACGGGACGTTG-3'; reverse: 5'-TAGATGTGAAGTGGAATTTC-3') Cui et al. [15], 10X PCR buffer (15 mM $MgCl_2$), 10 mM dNTPs, and 5 U Taq DNA polymerase.

The PCR cycling conditions included an initial denaturation step at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 45 seconds. A final extension step was performed at 72°C for 7 minutes.

Purification and Sequencing

PCR products were visualized on a 1.5% agarose gel stained with ethidium bromide. Amplified DNA fragments were purified using the ExoSAP-IT PCR Product Cleanup Kit (USB Corporation) according to the manufacturer's protocol. Sequencing was performed using an automated ABI 3730XL DNA Analyzer (Macrogen, Seoul, South Korea).

Sequence Analysis

The COX-3 gene sequences obtained from the samples were aligned with reference sequences from Camelus dromedarius in GenBank using the Clustal W algorithm in MEGA 11.0 software [*16*]. Genetic diversity parameters, including nucleotide diversity (π), haplotype diversity (Hd), the average number of nucleotide differences (K), and polymorphic sites (S), were calculated using DnaSP v5.10 [17].

Neutrality Tests

Neutrality tests, including Tajima's D and Fu's Fs [18;19], were conducted to evaluate deviations from neutral evolution. Tajima's D was used to identify low-frequency polymorphisms and detect population expansion or balancing selection. Fu's Fs test was applied for detecting population growth or purifying selection [20].

Phylogenetic Analysis

Phylogenetic relationships were assessed using a Neighbor-Joining tree constructed in MEGA X software (version 2020) [21]. The COX-3 sequences from Egyptian camel populations were compared with sequences from other camelids, including Camelus bactrianus (GenBank: MH109977.1), Lama glama (NC_012102.1), Bubalus bubalis (MT186736.1), and Bos taurus (MN714218.1).

3D Structure Prediction of the COX-3 Protein

The three-dimensional (3D) structure of the COX-3 protein was predicted using the Phyre2 protein modeling tool. The translated protein sequences were analyzed using ExPASy Translate (http://web.expasy.org/translate) to identify potential structural variations.

SNP Detection

Single nucleotide polymorphisms (SNPs) were identified using bioinformatics tools, including BioEdit v7.2.6, BLASTn, BLASTx, and NovelSNPer. Codon changes and their corresponding amino acid substitutions were analyzed to determine their impact on the protein function.

Results

Amplification and Sequencing of the COX-3 Gene

PCR amplification successfully yielded a 524-bp fragment of the mitochondrial COX-3 gene from all camel populations studied. Visualization of the amplified products on a 1.5% agarose gel confirmed the expected band size (Fig.1).

Haplotype Identification and Genetic Diversity

Sequenced fragments were aligned and compared to reference sequences of Camelus dromedarius available in the GenBank database. The analysis of the 524-bp COX-3 gene fragment revealed two haplotypes (Fig.2), distinguished by single nucleotide polymorphisms (SNPs) at positions 280 (C>T) and 325 (A>G) (Fig.3). The two haplotypes were submitted to GenBank under the accession numbers OP994029 (Haplotype 1) and OP994030 (Haplotype 2). Nucleotide composition showed that the A+T content was 51.15%, and C+G content was 48.85%. Haplotype diversity (Hd) was 0.667 ± 0.056, while nucleotide diversity (π) was 0.00254 ± 0.001. The average number of nucleotide differences (K) between haplotypes was 1.333 ± 0.063.

Among the three camel populations, Hd ranged from 0.376 to 0.797, and nucleotide diversity (π) ranged from 0.001 to 0.002. Haplotype 1 was more prevalent in Baladi and Sudani camels, while Haplotype 2 was dominant in the Maghrebi population.

Neutrality Tests

Tajima's D test produced a positive value (1.893), while Fu's Fs test resulted in a value of 1.530. These results suggest limited genetic diversity and a potential decline in population size or evidence of balancing selection, but neither value was statistically significant ($P > 0.1$).

Nucleotide BLAST (BLASTn)

The two detected haplotypes, Haplotype 1 (OP994029) and Haplotype 2 (OP994030), were subjected to BLASTn analysis to assess their similarity with other sequences in the GenBank database. The analysis revealed the following:

Both haplotypes displayed a high alignment score with Camelus dromedarius (KX554934.1) at 99.6% identity. Alignment with Camelus bactrianus (MH109977.1) indicated 93% similarity. Lower similarity was observed with Lama glama (NC_012102.1) at 84%, Bubalus bubalis (MT186736.1) at 80%, and Bos taurus (MN714218.1) at 79%. The sequences of the Egyptian haplotypes demonstrated the highest homology with dromedary camels, confirming their close phylogenetic relationship. However, notable divergence was observed when compared with species outside the Camelidae family, such as buffalo and cattle (Fig.4).

Phylogenetic Analysis

Phylogenetic relationships were inferred using a Neighbor-Joining tree. The sequences of Egyptian camels showed high similarity to Camelus dromedarius (KX554934.1) and moderate similarity to Camelus bactrianus (MH109977.1) with a genetic distance of 0.071. Egyptian haplotypes also displayed higher divergence from Lama glama (0.221–0.228), Bubalus bubalis (0.331–0.341), and

Bos taurus (0.344–0.345). These findings indicate that Egyptian camel haplotypes are closely related to other dromedary camels but exhibit significant genetic divergence from other species within the Camelidae family (Fig.2).

SNP Analysis and Protein Impact

The identified SNPs at positions 280 and 325 resulted in synonymous codon changes (CAC to CAT and CTA to CTG, respectively), producing no alteration in the encoded amino acids (Histidine at position 94 and Leucine at position 110). Functional conservation of the COX-3 protein was maintained, as predicted by bioinformatics analyses.

NovelSNPer

The analysis using Novel SNPer identified two single nucleotide polymorphisms (SNPs) in the COX-3 gene fragment. At position 280, a C-to-T transition altered the codon from CAC to CAT, encoding the same amino acid, Histidine. Furthermore, at position 325, an A-to-G transition resulted in a codon change from CTA to CTG, encoding Leucine without affecting its function. These variations were characterized as synonymous substitutions, meaning they did not result in changes to the encoded amino acids. The details of the SNPs are summarized in Table (1).

BLASTx

The BLASTx analysis of the COX-3 gene sequences revealed codon variations at positions 280 and 325, corresponding to synonymous substitutions. At position 280, a cytosine (C) to thymine (T) transition resulted in a codon change from CAC to CAT, both encoding the amino acid Histidine. Similarly, at position 325, an adenine (A) to guanine (G) transition altered the codon from CTA to CTG, both encoding Leucine. These substitutions did not affect the protein's amino acid sequence, indicating functional conservation of the COX-3 protein in Egyptian camels. Fig.5 illustrates these codon-level variations, highlighting their synonymous nature and lack of impact on protein structure or function.

Estimating Evolutionary Distances

The analysis of evolutionary distances between the two Egyptian camel haplotypes and various species revealed that Haplotype 1 and Haplotype 2 are closely related, with a genetic distance of 0.004. Both haplotypes exhibited minimal divergence from Camelus dromedarius (0.000–0.004), suggesting a recent common ancestor. When compared to Camelus bactrianus, the genetic distance was 0.071 for both Egyptian haplotypes, indicating a moderate level of divergence. In contrast, the distances between the Egyptian haplotypes and other species like Lama glama (0.221–0.228), Bubalus bubalis (0.331–0.341), and Bos taurus (0.344–0.345) were significantly greater, reflecting deeper evolutionary divergence. Table 2 summarizes these pairwise distances, confirming the close relationship between Egyptian camels and other dromedaries, while highlighting the evolutionary distinctiveness of camels relative to other species within the Camelidae family.

Phylogenetic constriction

The phylogenetic tree, shown in Fig.6**,** illustrates the evolutionary relationships of the two Egyptian camel haplotypes (Baladi, Sudani, and Maghrebi) based on the COX-3 gene sequence. The tree indicates that the Egyptian camels are most closely related to Camelus dromedarius (Arabian camel), with a genetic similarity of over 99%. This close relationship is further supported by the high homology of 93% with Camelus bactrianus (Mongolian camel). The tree also shows significant divergence between the Egyptian camels and species outside the Camelidae family, such as Lama glama (South American camelid) at 84%, Bubalus bubalis (water buffalo) at 80%, and Bos taurus (cattle) at 79%. These findings confirm that Egyptian camels share a relatively recent common ancestry with other dromedaries and exhibit a distinct evolutionary trajectory when compared to non-camelid species.

3D Structure Prediction of COX-3 Protein

The predicted 3D tertiary structure of the COX-3 protein from Egyptian camels, generated using Phyre2 software (Fig.7). The model, with 100% confidence, shows that the protein consists of 174 amino acids, with 68% of the structure forming α-helices. This high proportion of α-helices is typical for membrane-associated proteins like COX-3, which plays a crucial role in the mitochondrial respiratory chain. The structure also includes disordered regions (approximately 32%) (Fig.8), which may contribute to the protein’s flexibility and its interactions within the respiratory complex. The model confirms that the protein's functional architecture remains intact despite the synonymous nucleotide changes observed in the gene.

The amino acid sequence of the COX-3 protein from the Egyptian camel haplotypes (Haplotype 1 and Haplotype 2) illustrates in Fig.9 and submitted to the NCBI GenBank under the accession nos. WHO17330.1and WHO17331.1, respectively. the amino acid substitutions observed in the COX-3 gene at positions 280 (C>T) and 325 (A>G) do not alter the amino acid sequence (Histidine at position 94 and Leucine at position 110), indicating that the

protein's functionality is preserved despite the nucleotide changes. This preserved amino acid sequence supports the functional conservation of the COX-3 protein, indicating that these genetic variations do not impact the protein's structure or function.

Phyre2 Prediction of Amino Acid Localization

The localization of the two synonymous amino acid substitutions (C280T) in the predicted 3D structure of the COX-3 protein illustrates in Fig.10. The substitution at position 280 (C>T) results in the synonymous change from Histidine (94) to Histidine, and the substitution at position, with no alteration in the amino acid sequence. The locations of these residues are within the α-helix regions, indicating that these synonymous substitutions do not affect the overall structure or function of the protein. The wild-type amino acids (Histidine) is positioned in functionally important regions of the protein, suggesting that these changes have no significant impact on protein activity or stability.

While, Fig.11 shows the localization of the synonymous amino acid substitution at position 325 (A325G) in the COX-3 protein. This substitution alters the codon from CTA (Leucine) to CTG (Leucine), resulting in no change in the amino acid sequence. The substitution occurs within the α-helix region of the protein, which is essential for its structural integrity. The position of Leucine at residue 110 suggests that this synonymous mutation does not affect the protein's function or its role in the mitochondrial respiratory chain. The α-helical structure remains intact, and the protein's overall stability and function are preserved, confirming the minimal impact of this genetic variation.

Discussion

The present study aimed to evaluate the genetic diversity and phylogenetic relationships of Egyptian *Camelus dromedarius* populations by analyzing the mitochondrial COX-3 gene. Two haplotypes were identified based on synonymous single nucleotide polymorphisms (SNPs) at positions 280 (C>T) and 325 (A>G), which did not result in amino acid changes. The findings indicate strong conservation of the COX-3 gene, consistent with its essential role in cellular respiration and mitochondrial energy production. These results are in agreement with previous studies, which highlighted the evolutionary pressure to maintain the functional integrity of mitochondrial genes due to their involvement in oxidative phosphorylation [4, 22-23].

The observed haplotype diversity (Hd = 0.6671 ± 0.056) and nucleotide diversity (π = 0.00254 ± 0.001) are consistent with earlier research conducted on dromedary camels. For example, Almathen et al. [24]; Bahbahani et al. [25]; Manee et al. [11] reported similar levels of mitochondrial diversity in Arabian camels, attributing it to their adaptation to harsh desert environments and historical domestication bottlenecks. Low nucleotide diversity is a common observation in camels, likely reflecting their domestication history and their isolation in arid regions where gene flow is limited [3, 10]. Despite this limited diversity, the presence of two distinct haplotypes in the Egyptian populations suggests that genetic variation persists, enabling these camels to adapt to regional environmental pressures.

The environmental correlation observed in this study further reinforces the link between mitochondrial gene variations and environmental adaptation. Haplotype 1, predominant in Baladi and Sudani camels, was associated with inland arid regions characterized by extreme temperature fluctuations and limited water availability [26]. In contrast, Haplotype 2, which was more prevalent in Maghrebi camels, correlated with coastal regions where the climate is milder and more stable. This pattern aligns with studies by Bahbahani et al. [2]; Liu et al. [27] and Alaqeely et al. [28], which demonstrated that mitochondrial genes are often subjected to selective pressures imposed by environmental conditions. Mitochondrial haplotypes have been shown to influence energy metabolism efficiency, particularly under stress, which is a critical adaptation for camels thriving in extreme climates.

The phylogenetic analysis revealed a close relationship between the Egyptian haplotypes and other *Camelus dromedarius* sequences, as indicated by minimal genetic distances (0.000–0.004). This finding corroborates previous work by Abdel-Aziem et al. [29]; Ming et al. [10], which showed a high degree of mitochondrial homogeneity among dromedary camels due to their shared domestication origin and historical dispersal across arid regions. Moderate divergence from *Camelus bactrianus* (genetic distance = 0.071) was consistent with the estimated evolutionary separation of dromedaries and Bactrian camels approximately 2–3 million years ago [30; 3; 23]. Greater genetic distances were observed between the Egyptian camels and non-camelid species, such as *Lama glama*, *Bubalus bubalis*, and *Bos taurus*, which is consistent with earlier studies highlighting the distinct evolutionary lineage of the Camelidae family [4, 31-32].

The structural analysis of the COX-3 protein provided further insights into the functional conservation of this gene. The predicted 3D structure, generated using Phyre2, revealed that the protein is predominantly composed of α-helices, a

feature typical of mitochondrial membrane proteins involved in oxidative phosphorylation. Similar structural predictions have been reported in studies of mitochondrial proteins in other mammalian species, where α-helical regions play a critical role in maintaining protein stability and facilitating electron transport within the respiratory chain [5;11]. Importantly, the synonymous SNPs identified in this study were localized within α-helical regions, suggesting that evolutionary pressures have acted to preserve the structural integrity of the COX-3 protein. This aligns with findings from Mohandesan et al. [4], which demonstrated that mitochondrial genes, including COX-3, exhibit high structural conservation due to their central role in energy production.

Although synonymous mutations do not alter the encoded amino acid sequence, emerging evidence suggests that they can influence gene expression, mRNA stability, and translation efficiency, particularly in genes under strong selective pressure [2; 13]. The localization of the identified SNPs in conserved regions of the COX-3 protein raises the possibility that these mutations may have subtle functional effects, particularly under conditions of environmental stress where energy metabolism is critical. Functional studies, such as mRNA expression analysis and enzymatic activity assays, would be necessary to confirm this hypothesis.

This study contributes to the growing body of evidence on the genetic diversity and adaptive mechanisms of dromedary camels. However, it is important to acknowledge certain limitations. The reliance on a single mitochondrial marker, while informative, does not capture the full genetic complexity of camel populations. Previous research has shown that integrating nuclear DNA markers, such as microsatellites and genome-wide SNP arrays, provides a more comprehensive understanding of genetic variation and population structure [33; 34]. Additionally, the sample size, though sufficient for identifying major haplotypes, could be expanded to include more populations from diverse geographic regions. A larger dataset would allow for a more robust assessment of genetic differentiation and historical gene flow.

Conclusion

This study highlights the genetic diversity of Egyptian *Camelus dromedarius* populations and the functional conservation of the COX-3 gene. The identification of two haplotypes and their correlation with distinct environmental conditions underscores the role of mitochondrial genes in adaptive evolution. The structural stability of the COX-3 protein, as predicted by 3D modeling, further supports its evolutionary importance in energy metabolism. Future studies incorporating whole-genome analysis, nuclear markers, and functional experiments will provide a deeper understanding of the genetic adaptations that enable camels to thrive in extreme environments. These findings emphasize the need for conservation strategies to preserve the genetic resources of camel populations, ensuring their resilience in the face of environmental changes.

Acknowledgments

The authors gratefully acknowledge everyone who contributed to the completion of this study, including the cooperating camel farm owners.

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The study was approved by the Ethics of Medical Research Committee of the National Research Centre, Al Buhouth St., Dokki, Cairo, Egypt (Approval No. 12440723)."

Data availability

All obtained sequences have been submitted to GenBank (Acc.Numbers OP994029 - OP994030 and WHO17330.1- WHO17331.1) and the accession numbers are provided in the article.

Author Contributions

OE and SH. created the primary concept and original idea. HA, DM, and SA performed DNA extraction. SH and DM performed PCR and sequencing. SH, OE and DM analyzed data and wrote the manuscript. All authors approved the final manuscript.

TABLE 1. NovelSNPer detailed output file with ranscript variation per line

Position	Allele Change	Reference Allele	Variant Allele	Codon Change	Amino Acid	Effect
280	C > T	Histidine	Histidine	CAC > CAT	Histidine	Synonymous
325	A > G	Leucine	Leucine	CTA > CTG	Leucine	Synonymous

TABLE 2. Estimates of evolutionary divergence between sequences

	Hap 1	Hap 2	KX554934.1 *C. dromedarius*	MH109977.1 *C. bactrianus*	NC_012102.1 *Lama glama*	MT186736.1 *Bubalus bubalis*	MN714218.1 *Bos taurus*
Haplotype1	0.000						
Haplotype2	0.004	0.000					
KX554934.1 *C. dromedarius*	0.004	0.000	0.000				
MH109977.1 *C. bactrianus*	0.071	0.071	0.071	0.000			
NC_012102.1 Lama glama	0.221	0.228	0.228	0.249	0.000		
MT186736.1 Bubalus bubalis	0.331	0.341	0.341	0.348	0.291	0.000	
MN714218.1 Bos taurus	0.345	0.344	0.344	0.371	0.290	0.178	0.000

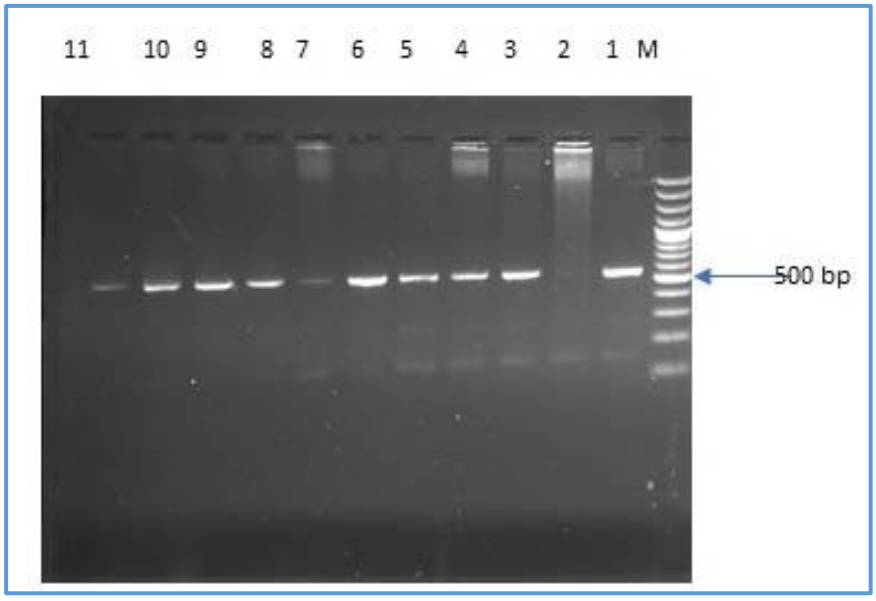


Fig. 1. Electrophoretic agarose ethidium bromide-stained gel showed PCR products for COX-3 gene. Lane 1: 100-bp DNA marker, Lanes 1-4 Baladi samples, lanes: 5-7 Sudani samples and 8-11 Maghrebi samples: 524-bp amplified fragments.

HAP1	HAP2
CCAGTGATGACGGGACGTTGTCCGAGAAAGCACA	CCAGTGATGACGGGACGTTGTCCGAGAAAGCACA
TTTCAAGGGCATCACACGCCTGCTGTCCAAAAAGG	TTTCAAGGGCATCACACGCCTGCTGTCCAAAAAGG
TCTACGATACGGAATAATCCTATTTATTGTGTCAG	TCTACGATACGGAATAATCCTATTTATTGTGTCAG
AGGTTTTATTTTTTACCGGATTCTTCTGAGCCTTTT	AGGTTTTATTTTTTACCGGATTCTTCTGAGCCTTTT
ACCACTCAAGCCTAGCCCCCACTCCCGAGCTAGGA	ACCACTCAAGCCTAGCCCCCACTCCCGAGCTAGGA
GGATGCTGACCTCCCACCGGCATCCACCCCTTAAA	GGATGCTGACCTCCCACCGGCATCCACCCCTTAAA
CCCGCTAGAAGTCCCTCTTCTCAATACCTCTGTCCT	CCCGCTAGAAGTCCCTCTTCTCAATACCTCTGTCCT
ATTAGCCTCCGGAGTCTCAATCACCTGAGCCCACC	ATTAGCCTCCGGAGTCTCAATCACCTGAGCCCATC
AGCCTGATGGAAGGCAACCGTGCCCATATACTCC	ACAGCCTGATGGAAGGCAACCGTGCCCATATACT
AGGCCCTATTTATTACGATTGCCCTGGGACTATAT	CCAGGCCCTGTTTATTACGATTGCCCTGGGACTAT
TTCACGCTACTCCAAGCATCAGAGTACTACGAAGC	ATTTCACGCTACTCCAAGCATCAGAGTACTACGAA
ACCCTTCACAATCTCAGACGGTGTTTATGGGTCCA	GCACCCTTCACAATCTCAGACGGTGTTTATGGGTC
CCTTCTTTGTAGCCACTGGATTCCATGGGCTACAT	CACCTTCTTTGTAGCCACTGGATTCCATGGGCTAC
GTTATTATTGGCTCCACTTTCCTGACTGTATGCTTC	ATGTTATTATTGGCTCCACTTTCCTGACTGTATGCT
CTACGACAACTGAAATTCCACTTCACATCTA	TCCTACGACAACTGAAATTCCACTTCACATCTA

Fig. 2. The nucleotide sequence of 524-bp amplified fragment of COX3 gene SNPs at positions 280 and 325 in Bold

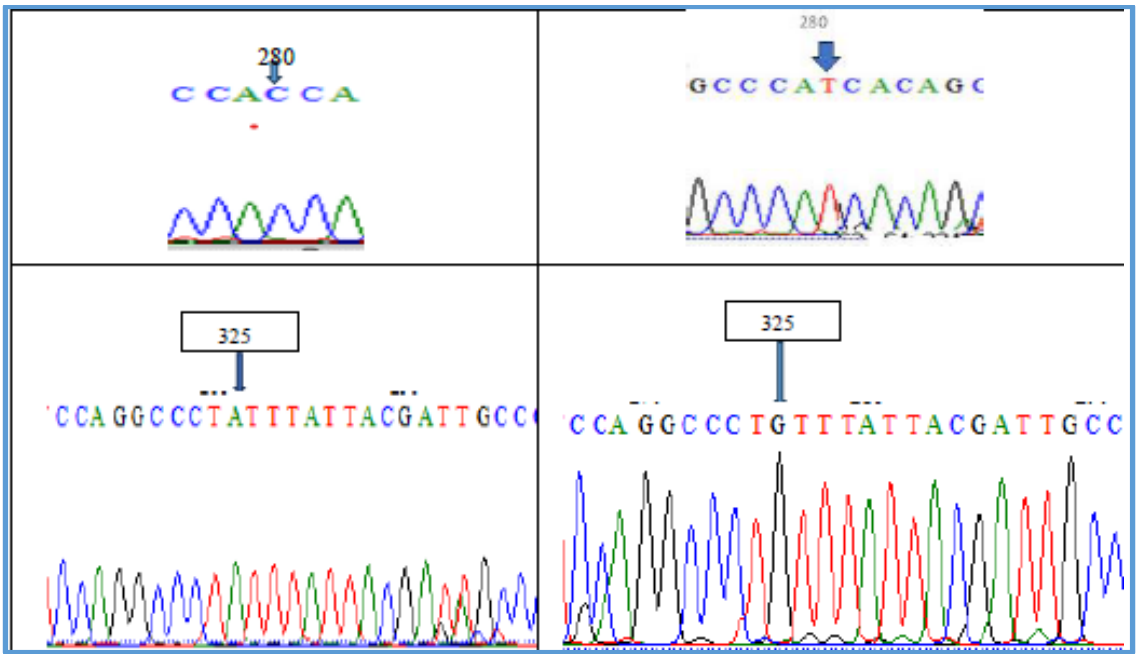
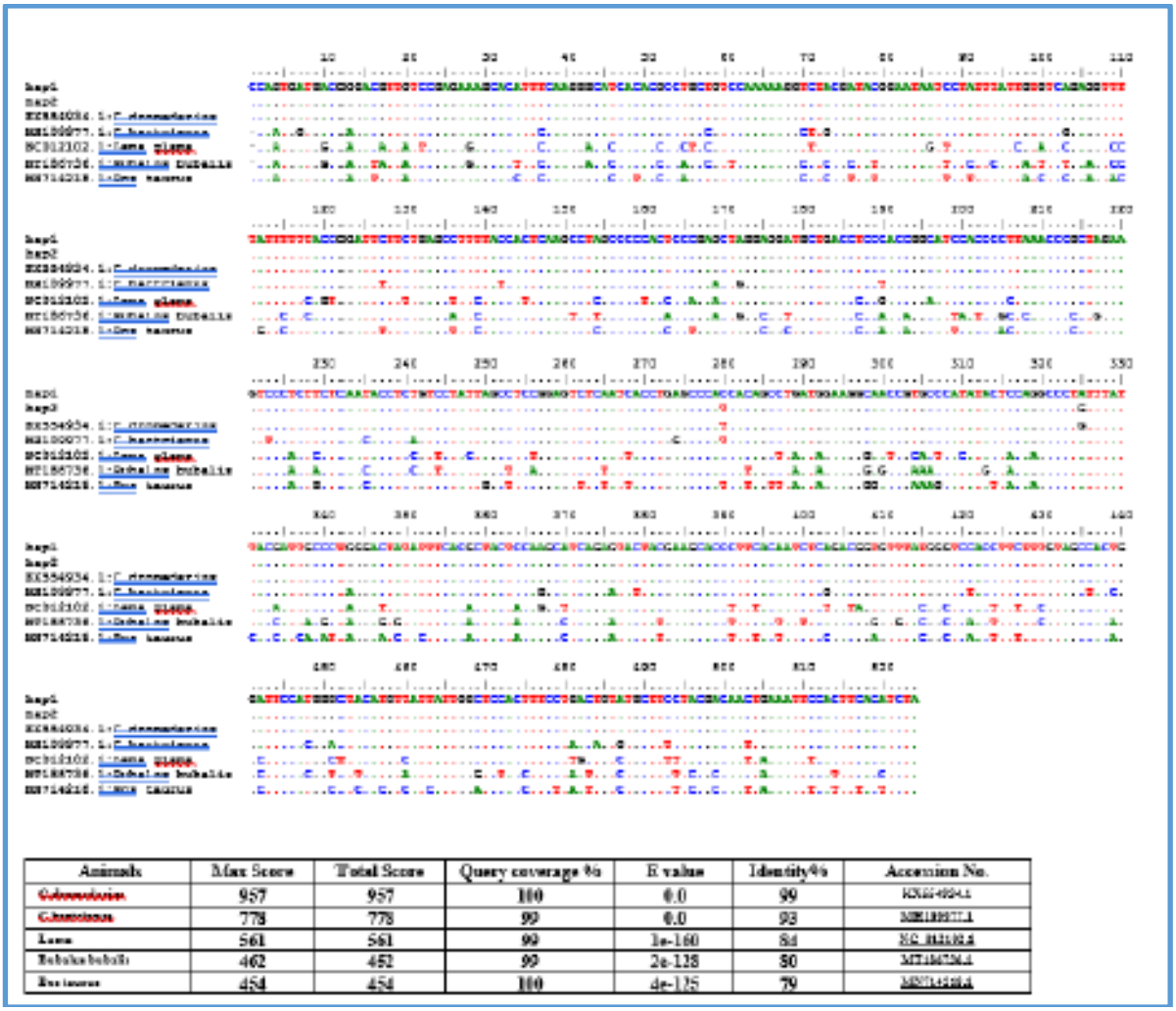


Fig. 3. The genotype AT **of** *COX-3* **gene with A/G nucleotide at position**



Animals	Max Score	Total Score	Query coverage %	E value	Identity%	Accession No.
Camelus dromedarius	957	957	100	0.0	99	KX654934.1
Camelus bactrianus	778	778	99	0.0	93	MK189977.1
Equus	561	561	99	1e-160	84	NC_013103.1
Bubalus bubalis	462	462	99	2e-128	80	MT186736.1
Bos taurus	454	454	100	4e-125	79	MH714218.1

Fig. 4. Illustrates the alignment of query sequences from Egyptian camels with the top five species identified in the BLASTn search.

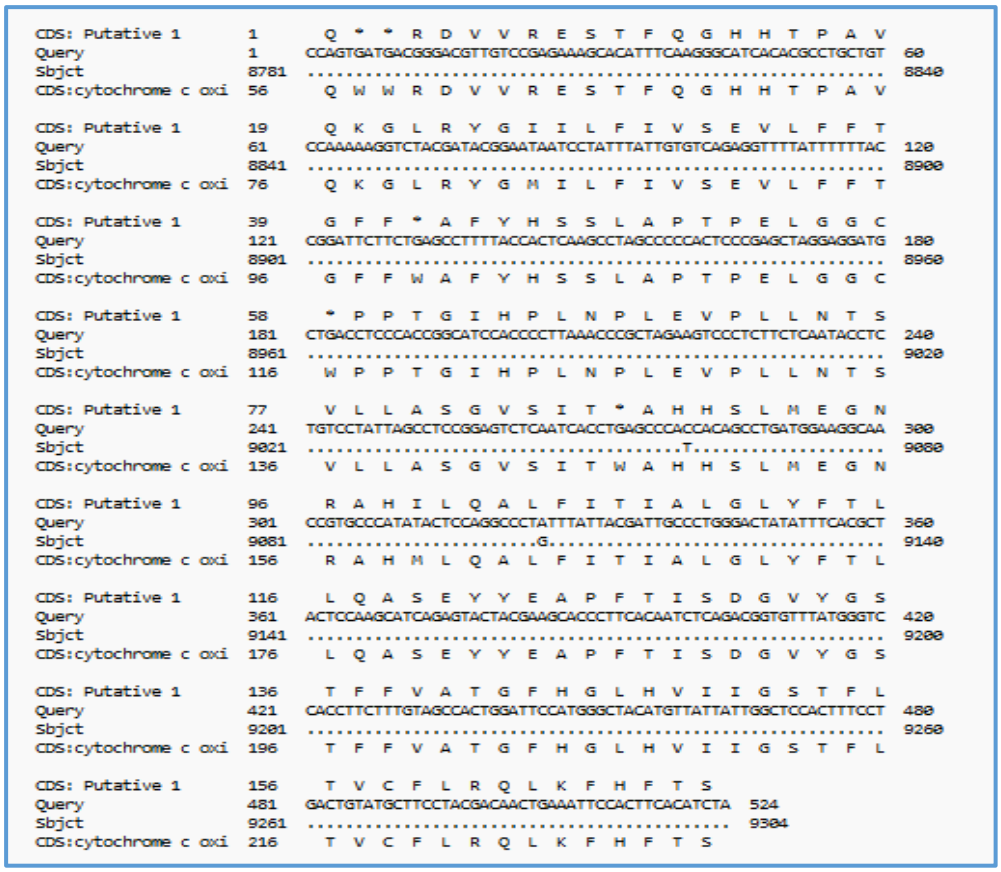


Fig. 5. Detailed output of codons showing variations

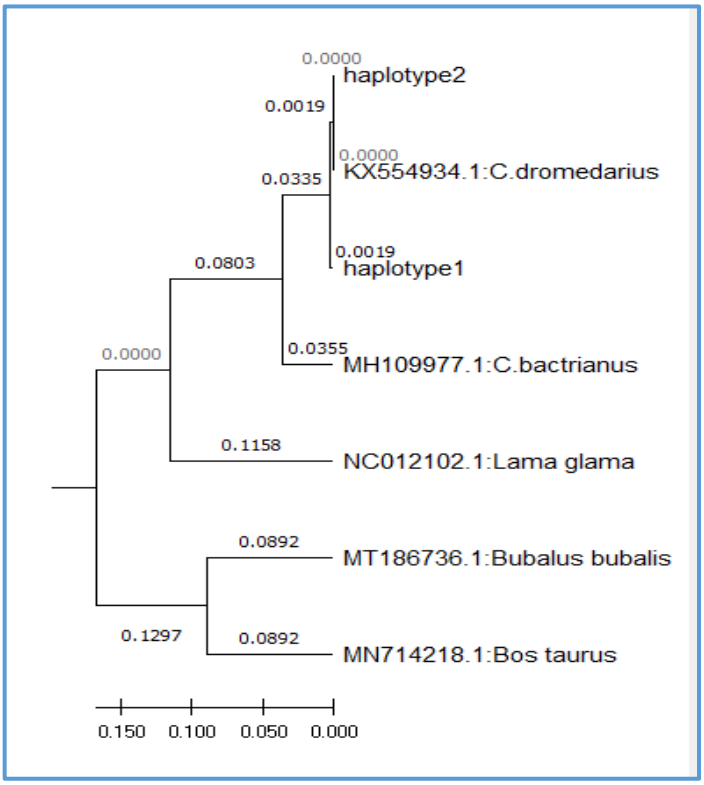


Fig. 6. The phylogenetic tree displays the genetic distances among the three Egyptian camel breeds (Maghrebi and Fellahi) and other organisms based on the COX-3 gene sequence

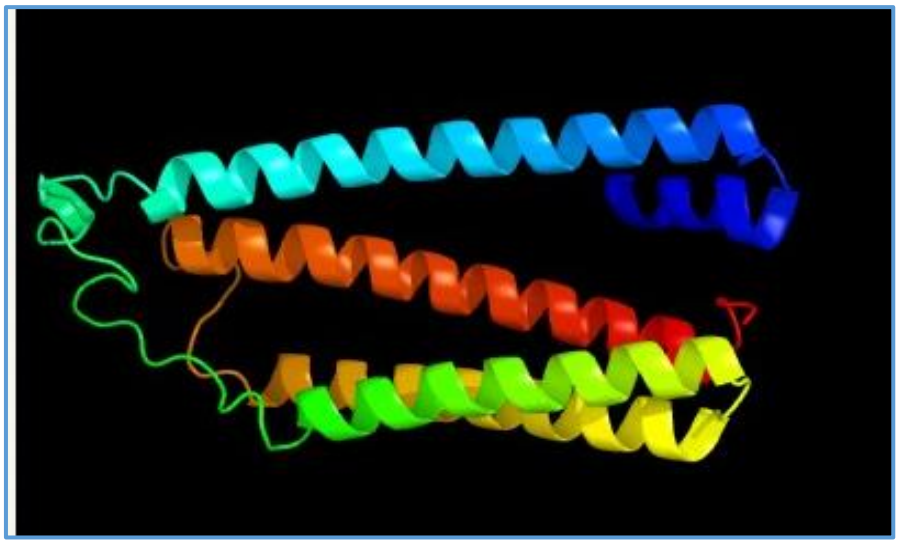


Fig. 7. The predicted 3D tertiary structure of the Camel COX-3 reared in Egypt

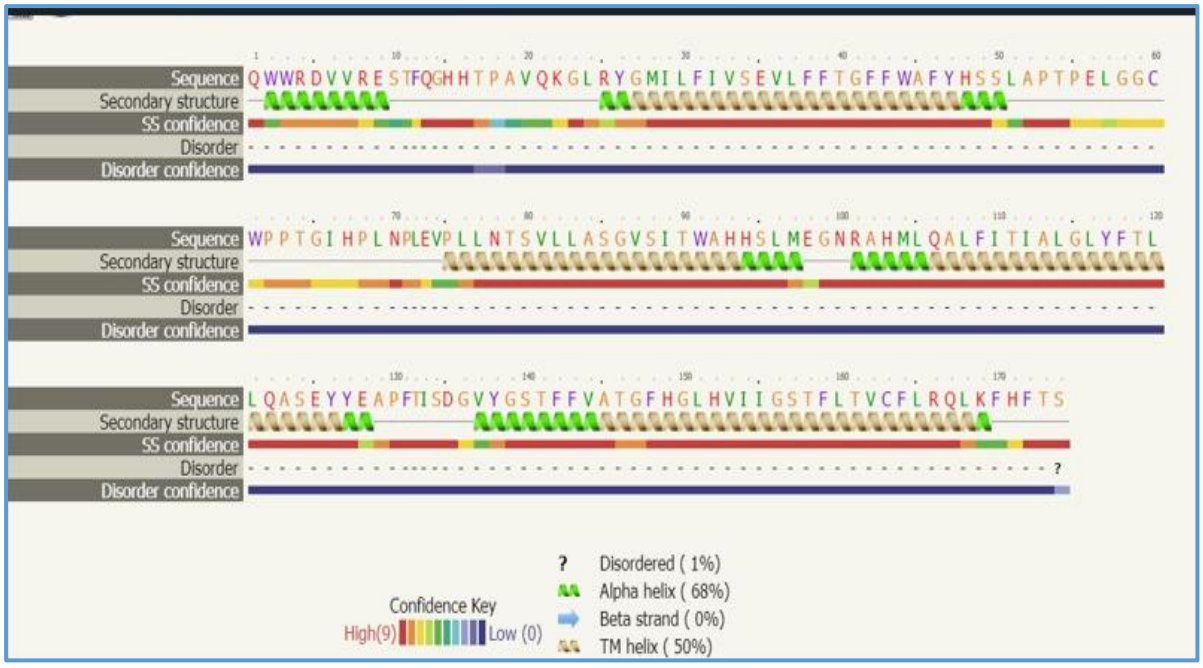


Fig. 8. The secondary structure of the 3D model of COX-3 protein consists of α-helix (green), beta strands (blue), and disordered regions (?).

Hep1	Hep2
QWWRDVVRESTFQGHHTPAVQKGLRYGMILFIV	QWWRDVVRESTFQGHHTPAVQKGLRYGMILFIV
SEVLFFTGFFWAFYHSSLAPTPELGGCWPPTGIHP	SEVLFFTGFFWAFYHSSLAPTPELGGCWPPTGIHP
LNPLEVPLLNTSVLLASGVSITWAHHSLMEGNRA	LNPLEVPLLNTSVLLASGVSITWAHHSLMEGNRA
HMLQALFITIALGLYFTLLQASEYYEAPFTISDGV	HMLQALFITIALGLYFTLLQASEYYEAPFTISDGV
YGSTFFVATGFHGLHVIIGSTFLTVCFLRQLKFHF	YGSTFFVATGFHGLHVIIGSTFLTVCFLRQLKFHF
TS	TS

Fig. 9. Shows the amino acid for COX-3 protein in Egyptian camels

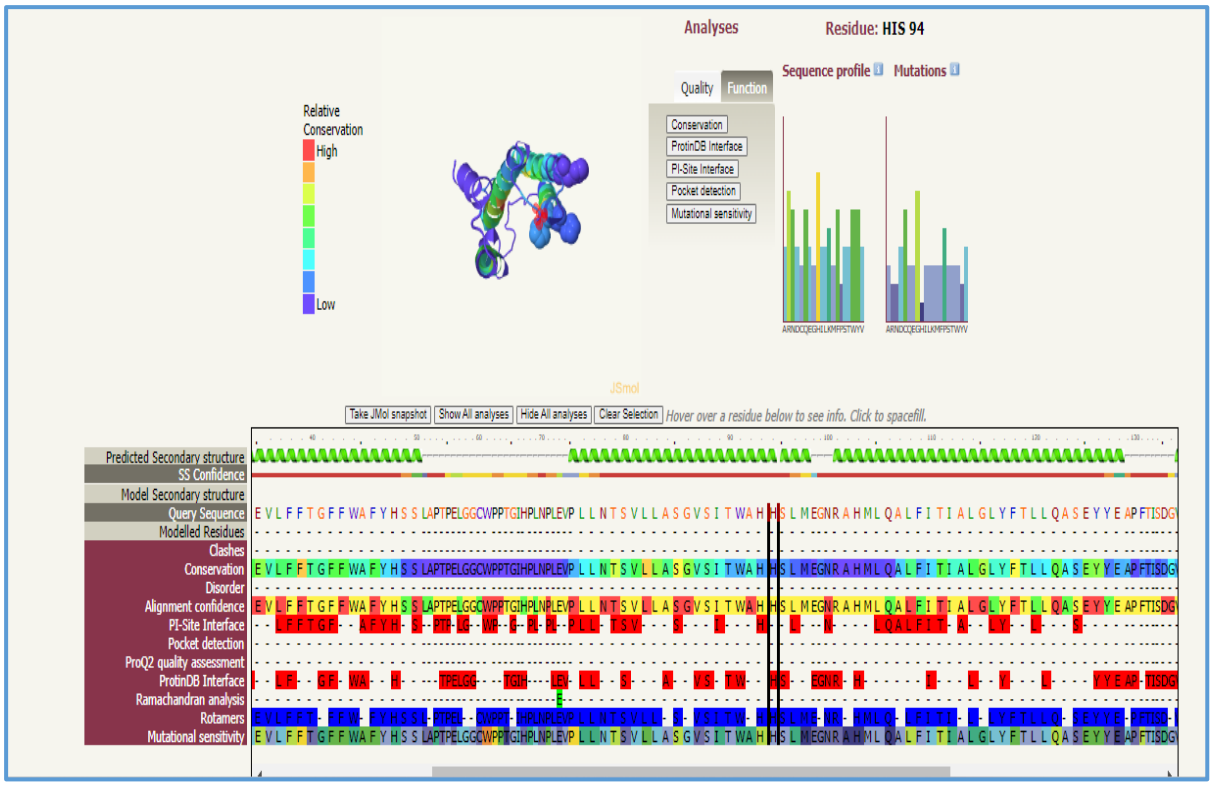


Fig. 10. The result of the phyre2 investigator showed the wild amino acid Histidine located in a α-helix region.

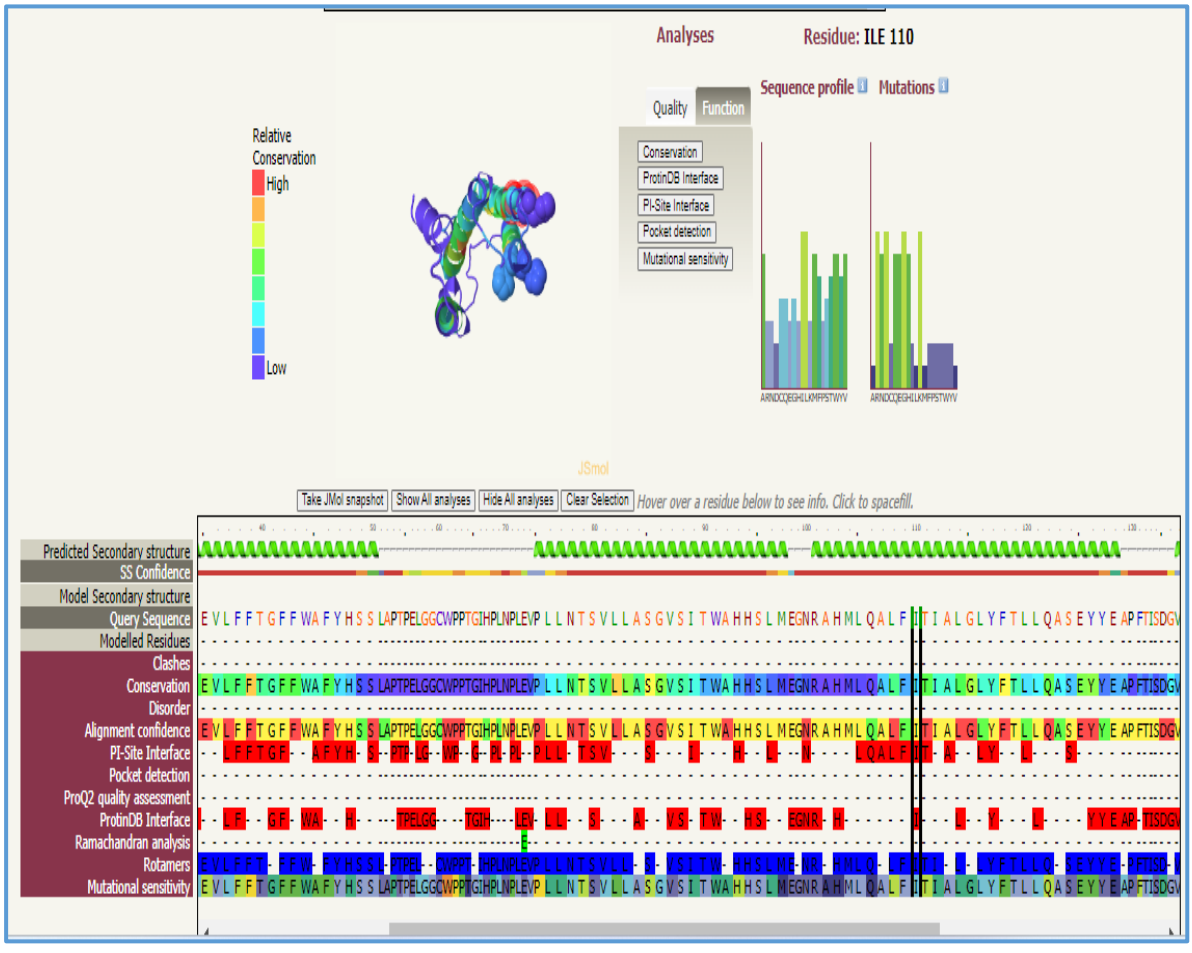


Fig. 11. The result of the phyre2 investigator showed the wild amino acid Leucine located in a α-helix region.

References

1. Faye, B. How many large camelids in the world? A synthetic analysis of the world camel demographic changes. *Pastoralism* **10**,25(2020). https://doi.org/10.1186/s13570-020-00176-z
2. Bahbahani, H., Musa, H.H., Wragg, D., Shuiep, E.S., Almathen, F. and Hanotte, O. Genome diversity and signatures of selection for production and performance traits in dromedary camels. *Frontiers in genetics*, **10**, p.893 (2019). doi: 10.3389/fgene.2019.00893.
3. Ming, L., Yi, L., Sa, R., Wang, Z.X., Wang, Z. and Ji, R. Genetic diversity and phylogeographic structure of Bactrian camels shown by mitochondrial sequence variations. *Animal Genetics*, **48**(2),217-220 (2017). https://doi.org/10.1111/age.12511
4. Mohandesan, E., Fitak, R.R., Corander, J., Yadamsuren, A., Chuluunbat, B., Abdelhadi, O., Raziq, A., Nagy, P., Stalder, G., Walzer, C. and Faye, B. Mitogenome sequencing in the genus Camelus reveals evidence for purifying selection and long-term divergence between wild and domestic Bactrian camels. *Scientific reports*, **7**(1),.9970 (2017). doi:10.1038/s41598-017-08995-8.
5. Ali, A., Baby, B. and Vijayan, R. From desert to medicine: a review of camel genomics and therapeutic products. *Frontiers in genetics*, **10**, 17 (2019). doi:10.3389/fgene.2019.00017
6. Al-Swailem, A.M., Shehata, M.M., Abu-Duhier, F.M., Al-Yamani, E.J., Al-Busadah, K.A., Al-Arawi, M.S., Al-Khider, A.Y., Al-Muhaimeed, A.N., Al-Qahtani, F.H., Manee, M.M. and Al-Shomrani, B.M., Sequencing, analysis, and annotation of expressed sequence tags for Camelus dromedarius. *PLoS One*, **5(**5), e10720 (2010).
7. Bardakci, F., Al-Subaie, S.H.M., Badraoui, R., Adnan, M. and Siddiqui, A.J. Molecular Characterization of Hard Ticks Infesting Camels in the Northern Region of Saudi Arabia Using the Barcoding Gene, Mitochondrial Cytochrome oxidase subunit I. *Life*, **13(**7),1535(2023).https://doi.org/10.3390/life13071535
8. Faye, B., G. Konuspayeva, E. M. Knoll, and P. A. Burger. "Camels in Asia and North-Africa-Interdisciplinary Perspectives on their Past and Present Significance." (2012).
9. Wu, H., Guang, X., Al-Fageeh, M.B., Cao, J., Pan, S., Zhou, H., Zhang, L., Abutarboush, M.H., Xing, Y., Xie, Z. and Alshanqeeti, A.S. "Camelid genomes reveal evolution and adaptation to desert environments." *Nature communications* **5**(1)5188 (2014).https://doi.org/10.1038/ncomms6188
10. Ming, L., Siren, D., Hasi, S., Jambl, T. and Ji, R., 2022. Review of genetic diversity in Bactrian camel (Camelus bactrianus).*Animal Frontiers* **12**(4) 20-29 (2022).
11. Manee, M.M., Al-Shomrani, B.M. and Alqahtani, F.H., Mitochondrial DNA of the Arabian Camel Camelus dromedarius. *Animals*, **14**(17).2460(2024), doi: 10.3390/ani14172460.
12. Bailey, S.F., Alonso Morales, L.A. and Kassen, R. Effects of Synonymous Mutations beyond Codon Bias: The Evidence for Adaptive Synonymous Substitutions from Microbial Evolution Experiments. *Genome biology and evolution* **13**(9):evab141 (2021) doi: 10.1093/gbe/evab141.
13. Oelschlaeger P. Molecular Mechanisms and the Significance of Synonymous Mutations. *Biomolecules*. **14**(1),132(2024) https://doi.org/10.3390/biom14010132
14. Wajid, A., Wasim, M., Yaqub, T., Firyal, S., Tayyab, M., Siddique, S. and Hussain, T. Assessment of genetic diversity in Balochi and Rakhshani sheep breeds of Balochistan using microsatellite DNA markers. *J. Anim. Pl. Sci*., 24: 1348-1354(2014)..
15. Cui, P., Ji, R., Ding, F., Qi, D., Gao, H., Meng, H., Yu, J., Hu, S. and Zhang, H. A complete mitochondrial genome sequence of the wild two-humped camel (camelus bactrianus ferus): an evolutionary history of camelidae. *BMC Genomics***,** **3**, 241(2007).
16. Tamura, K., Stecher, G., Peterson, D., Filipski A. and Kumar, S. MEGA6: molecular evolutionary genetics analysis version 6.0 *Mol Biol Evol*. **30**(12):2725–2729. (2013).
17. Librado, P. and Rozas, J. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496-2497(2009).
18. Tajima, F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics.* **123**, 585–95(1989). .https://doi:10.1101/gad.3.11.1801.
19. Fu, Y.X. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics.* **147**,915–25(1997). .doi: 10.1093/genetics/147.2.915.
20. Al-Jumaili, A.S., Boudali, S.F., Kebede, A., Al-Bayatti, S.A., Essa, A.A., Ahbara, A., Aljumaah, R.S., Alatiyat, R.M., Mwacharo, J.M., Bjørnstad, G. and Naqvi, A.N. The maternal origin of indigenous domestic chicken from the Middle East, the north and the horn of Africa. *BMC Genetics.* **21**, 1-16(2020). doi: 10.1186/s12863-020-0830-0
21. Kumar, S., Stecher, G.and Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33** 1870-1874(2016).https://doi.org/10.1093/molbev/msw054
22. Ali, A., Rehman, M.U., Ahmad, S.M., Mehraj, T., Hussain, I., Nadeem, A., Mir, M.U.R .and Ganie, S.A. In Silico Tools for Analysis of Single-Nucleotide Polymorphisms in the Bovine Transferrin Gene. *Animals (Basel).* **12**(6),693(2022). doi: 10.3390/ani12060693
23. Casanova, A., Wevers, A., Navarro-Ledesma, S. and Pruimboom, L. Mitochondria: It is all about energy. *Frontiers in physiology*, **14**, 1114231 (2023). doi: 10.3389/fphys.2023.1114231
24. Almathen, F., Bahbahani, H., Elbir, H., Alfattah, M., Sheikh, A. and Hanotte, O. Genetic structure of

Arabian Peninsula dromedary camels revealed three geographic groups. *Saudi J Biol Sci.* **29**(3),1422-1427(2021). doi: 10.1016/j.sjbs.11.032.

25. Bahbahani, H. and Almathen, F. Homogeneity of Arabian Peninsula dromedary camel populations with signals of geographic distinction based on whole genome sequence data. *Sci Rep*.,**12**(1),130(2022). doi:10.1038/s41598-021-04087-w
26. Al Askar, H., Alhajeri, B.H., Almathen, F. and Alhaddad, H. Genetic Diversity and Population Structure of Dromedary Camel-Types. *J. Hered*, 111, 405-413(2020).
27. Liu, Y., Zhao, S., Zhang, J. and et al. Phylogenetic analysis of mitochondrial genome sequences from domestic Bactrian camels and their relatives. *Genes (Basel)*, **10**(9),719(2019).
28. Alaqeely, R., Alhajeri, B.H., Almathen, F. and Alhaddad, H. Mitochondrial Sequence Variation, Haplotype Diversity, and Relationships Among Dromedary Camel-Types. *Front Genet*. **12**:723964(2021). doi: 10.3389/fgene.2021.723964.
29. Abdel-Aziem, S.H., Mabrouk, D.M., Abd El-Kader, H.A., Alam, S.S.and Othman, O.E. Genetic similarity and diversity among three camel populations reared in Egypt. *J Genet Eng Biotechnol* **20**(1), 154(2022). doi: 10.1186/s43141-022-00435-z.
30. Di Lorenzo, P., Lancioni, H., Ceccobelli, S., Curcio, L., Panella, F.and Lasagna, E. Uniparental genetic systems: a male and a female perspective in the domestic cattle origin and evolution. *Electron J Biotechnol*., **23**, 69–78(2016).
31. Charruau, P. Insights from evolutionary history and population genetics for domestic and wildlife conservation – cases of the Old-World camelids and cheetahs. Thesis, *University of Veterinary Medicine*, Vienna (Austria) (2012).
32. Beati, L., Nava, S., Burkman, E.J., Barros-Battesti, D.M., Labruna, M.B., Guglielmone, A.A., Cáceres, A.G., Guzmán-Cornejo, C.M., León, R., Durden, L.A. and Faccini, J.L. Amblyomma cajennense (Fabricius, 1787) (Acari: Ixodidae), the Cayenne tick: phylogeography and evidence for allopatric speciation. *BMC Evol Biol*., **9**, 1-20(2013). doi: 10.1186/1471-2148-13-267.
33. Al-Soudy, A., El-Sayed, A., El-Itriby, H. and Hussein, E. 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 & Endangered Species*, **6**(1),1-8 (2018).doi:10.4172/2332- 2543.S2-001
34. Mutery, A.A., Rais, N., Mohamed, W.K. and Abdelaziz, T. Genetic diversity in casein gene cluster in a dromedary camel (C. dromedarius) Population from the United Arab Emirates. *Genes*, **12**(9),1417(2021). doi: 10.3390/genes12091417.

التوصيف الجينى الحيوى والرؤى الوظيفية الحاسوبيه للتنوع الجينى للميتوكنودريا (COX-3) في الإبل المصرية (*Camelus dromedarius*)

سكينة حسانين عبد العظيم ، داليا ممدوح مبروك، هبه احمد محمد عبد القادر، سالى احمد سعيد علام و عثمان المهدى عثمان

قسم بيولوجيا الخلية، المركز القومى للبحوث، دقى، جيزة، مصر.

الملخص

ان دراسة التنوع الجيني للإبل المصرية أمرا بالغ الاهمية لتقيم قدرتها على التكيف مع البيئات الصحراوية القاسية وتوجيه استراتيجيات الحفاظ عليها. حللت هذه الدراسة التباين في ثلاث سلالات من الابل (***Camelus dromedarius***) وهى البلدي، السوداني و المغربي باستخدام جين الميتوكنودريا (COX-3) حيث تم استخلاص الحمض النووى من ٩٠ عينة دم (٣٥ بلدي، ٣٠ سوداني، ٢٥ مغربي) كشفت النتائج عن وجود نمطين وراثيين (C>T)عند الوضع ٢٨٠و عند ٣٢٥ (A>G) وسجلت هذه الانماط في بنك الجينات برقمي دخول OP994029و OP994030مع الحفاظ على تسلسل الاحماض الامينية (الهيستيدين ٩٤ و الليوسين ١١٠) وسجلت تسلسل الاحماض الامينية برقمي WHO17330.1 و WHO17331.1. تشير النتائج الى النمط الوراثي الاول سائد في الإبل البلدي والسوداني (المناطق الحارة والجافة) بينما النمط الوراثي الثاني منتشر في الإبل المغربي (المناطق الساحلية المعتدلة) مما يشير الى تكيفات جينية مرتبطة بالظروف البيئية: تؤكد هذه النتائج على اهمية التنوع الجيني للميتوكوندريا في تحمل الاجهاد المناخي وتسلط الضوء على الحاجة الى استراتيجيات للحفاظ على هذه السلالات لضمات تكيفها مع التغيرات المناخية المستقبلية.

الكلمات الدالة: الجمل العربى (*Camelus dromedarius*)، COX-3، التباين الجينى، التكيف البيئى، تعدد الاشكال المفردة (SNPs).