Preliminary assessment of mitochondrial and functional nuclear gene variation in Kuri cattle (Lake Chad Basin)

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Abstract: Kuri cattle of the Lake Chad Basin are a geographically restricted African cattle population characterised by ecological adaptation and a complex taurine–zebu genomic background. This study characterised mitochondrial DNA diversity and selected functional nuclear gene variation in eight Kuri cattle (six bulls and two cows). A mitochondrial D-loop fragment was sequenced to assess maternal lineage diversity, while nuclear loci associated with milk protein composition (*CSN1S1*, *CSN2*, *CSN1S2*, *CSN3* and *LGB*), lipid metabolism (*DGAT1*) and thermoregulation (*PRLR/SLICK*) were analysed by PCR and Sanger sequencing. Mitochondrial analysis revealed heterogeneous D-loop sequence patterns among the examined individuals, indicating more than one maternal lineage within the sample. Nuclear genotyping showed polymorphism at several milk protein loci, including *CSN2*, where both A1 and A2 alleles were detected. The selected *CSN3* SNP was treated as a single biallelic locus. The *DGAT1* locus also showed allelic variation, whereas all screened *SLICK*-associated *PRLR* variants were homozygous wild type. Although based on a limited number of animals, the results indicate that functional genetic variability persists in this locally adapted cattle population and provide baseline molecular data for future conservation and breeding studies.

Keywords: D-loop, casein polymorphism, *DGAT1 K232A, SLICK* locus, conservation genetics.

Introduction

The ongoing erosion of livestock genetic diversity driven by climate change, production intensification and demographic pressure highlights the importance of conserving locally adapted animal genetic resources (Rischkowsky and Pilling, 2007; Groeneveld et al., 2010). Indigenous cattle populations maintained under marginal or extreme environmental conditions represent valuable reservoirs of functional genetic variation associated with resilience, metabolic flexibility and environmental adaptation (Boettcher et al., 2015). The preservation and molecular characterisation of such populations are therefore essential both for sustainable agriculture and for long-term breeding strategies.

The Kuri cattle breed of the Lake Chad Basin constitutes one of the most distinctive and geographically restricted cattle populations in Africa. Also known under several local names, including White Lake Chad, Boudouma and Bahari, Kuri cattle are characterised morphologically by the absence of a hump and by their large, bulbous horns (Figure 1) (Malbrant et al., 1947; Tawah et al., 1997).

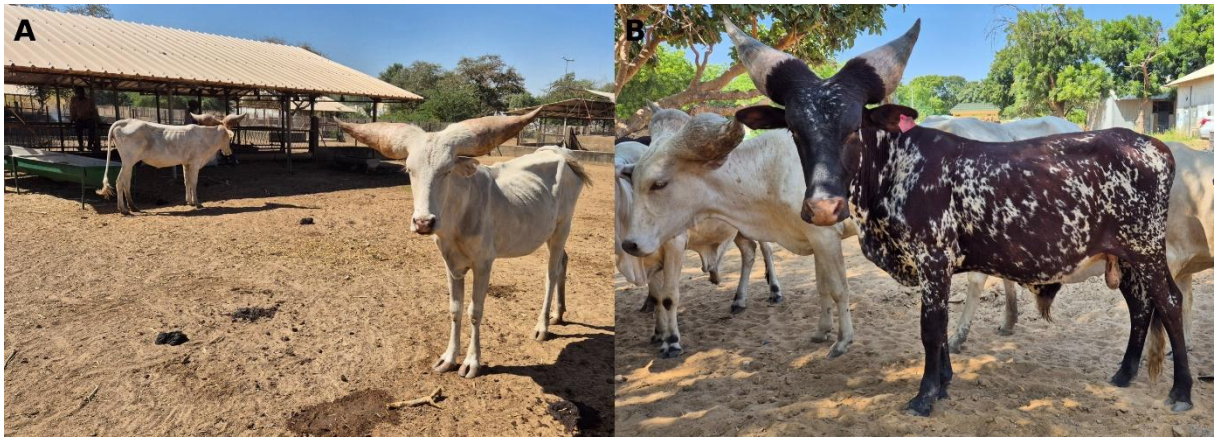

Figure 1 Representative Kuri cattle from the Lake Chad Basin (Republic of Chad)
(A) White Kuri cattle illustrating the typical horn morphology and humpless body conformation (B) Kuri bull showing the characteristic bulbous horns and spotted coat pattern. Photographs taken during field sampling in 2025.

Although traditionally classified among African taurine cattle based on phenotype, molecular studies have demonstrated a more complex genomic background reflecting historical admixture between taurine and zebu lineages (Meghen et al., 2006; Souvenir Zafindrajaona et al., 1999; Hanotte et al., 2002). In traditional pastoral systems of the Lake Chad Basin, Kuri cattle are maintained as a multipurpose breed used for milk, meat and draught power (Tawah et al., 1997).

The breed is distributed around Lake Chad across the border regions of Chad, Cameroon, Niger and Nigeria, where animals are maintained under traditional pastoral systems closely linked to seasonal water dynamics (Tawah et al., 1997; Blench, 1999). Kuri cattle display behavioural and morphological traits consistent with long-term adaptation to a semi-aquatic production environment, including frequent movement between islands and utilisation of shallow-water grazing areas (Epstein, 1971; Tawah et al., 1997). Despite these adaptive features, the population is considered vulnerable due to the progressive shrinkage of Lake Chad, recurrent drought periods and increasing crossbreeding with zebu cattle (Scherf and Pilling, 2015; Hanotte et al., 2002; Pitt et al., 2019).

Previous genetic investigations have primarily focused on neutral markers and genome-wide ancestry patterns, demonstrating admixture while confirming the persistence of taurine components within the breed. These studies indicate that Kuri cattle have a complex genetic background, with affinities to African taurine cattle as well as evidence of introgression from indicine/zebu lineages. In this respect, Kuri cattle can be considered genetically related to other indigenous African cattle populations that retain taurine ancestry, while also showing the influence of zebu admixture. However, comparatively limited information is available regarding functional genetic variation in loci directly related to production traits and environmental adaptation (Decker et al., 2014). Polymorphisms in milk protein genes have been widely investigated in cattle because of their influence on milk composition, processing properties and technological value (Grădinaru et al., 2018). Similarly, variants in genes associated with lipid metabolism and thermoregulation contribute to production efficiency and environmental resilience under extensive management conditions. One such example is the prolactin receptor gene (*PRLR*), where mutations associated with the so-called *SLICK* hair phenotype have been shown to improve heat tolerance in certain tropical cattle populations (Littlejohn et al., 2014; Boettcher et al., 2015). In addition to nuclear loci, mitochondrial DNA is widely used to study maternal lineages and historical population processes. In cattle and other livestock species, mitochondrial markers have frequently been applied to investigate genetic diversity, phylogeographic patterns and population history (Lenstra et al., 2014). Analysing mitochondrial sequences together with functional nuclear genes therefore allows a combined assessment of both maternal ancestry and variation in production-related loci.

In the present study, we analysed variation in several functional nuclear genes (*CSN1S1*, *CSN2*, *CSN1S2*, *CSN3*, *LGB*, *DGAT1* and *PRLR/SLICK*) together with mitochondrial D-loop sequences in a small sample of Kuri cattle from the Lake Chad Basin. Given the ecological isolation and demographic vulnerability of this population, we aimed to explore whether functional genetic variability is still detectable at these loci. These findings provide preliminary molecular insight into the genetic variation of Kuri cattle and may contribute to future research and conservation strategies aimed at preserving this locally adapted African cattle population.

Materials and methods

Sample collection

Hair samples were collected in 2025 from eight Kuri cattle ($n = 8$) in the Lake Chad region, Republic of Chad, from animals kept at the Institut de Recherche en Élevage pour le Développement (IRED; Livestock Research Institute for Development). The sampled animals were originally purchased by the institute from local pastoralists and maintained under traditional management conditions in the Lake Chad area. The sampled animals included six bulls and two cows originating from different herds owned by independent farmers. Information on pedigree relationships among the sampled individuals was not available.

Sampling was conducted under field conditions within traditional pastoral production systems. Hair samples were obtained non-invasively from the tail switch, stored in DNA/RNA Shield stabilising solution (Zymo Research, USA), and transported for molecular genetic analysis.

Ethics statement

Hair sampling was conducted non-invasively from the tail switch of live animals and did not involve surgical intervention, tissue biopsy, prolonged restraint or procedures associated with more than minimal discomfort. The procedure was limited to the collection of hair material and was carried out in accordance with internationally accepted animal welfare principles for non-invasive biological sampling.

The study was conducted in collaboration with the Institut de Recherche en Élevage pour le Développement (IRED), Republic of Chad. Sampling was performed under field conditions with the cooperation of local staff and animal handlers. The sampling protocol was reviewed and approved by the Institutional Animal Welfare Committee of the Hungarian University of Agriculture and Life Sciences, MATE Szent István Campus (approval number: MATE SzIC MÁB H/1467-1/2024).

Collected hair samples were stored in DNA/RNA Shield stabilising solution and transported to Hungary for molecular genetic analysis following institutional procedures applicable to non-invasive animal-derived biological samples intended for research purposes.

Genomic DNA extraction

Genomic DNA was extracted from the collected tail hair samples using the MagCore Genomic DNA Tissue Kit (RBC Bioscience Corp., Taiwan) according to the manufacturer's protocol. DNA was eluted in 60 µL nuclease-free water and stored at −20 °C until further analysis. Initial DNA purity was assessed using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific, USA). Samples exhibiting A260/A280 ratios between 1.8 and 2.2 were considered suitable for downstream applications. DNA concentration was subsequently determined using a Qubit™ fluorometer (Thermo Fisher Scientific, USA) with the dsDNA High Sensitivity Assay. DNA integrity and fragment size distribution were evaluated using an Agilent TapeStation system (Agilent Technologies, USA). Fluorometric quantification and fragment analysis were performed at Eurofins Biomi Kft. (Gödöllő, Hungary). DNA samples were normalised to a working concentration of 15 ng/µL for PCR amplification.

Mitochondrial DNA analysis

A fragment of the mitochondrial genome was amplified using the previously described primer pair AF22 and AF23 targeting the mitochondrial D-loop region (Putri et al., 2019). The primer sequences were AF22 forward 5′-GCGTACGCAATCTTACGATCA-3′ and AF23 reverse 5′-ATGCAGTTAAGTCCAGCTAC-3′. PCR amplification yielded an approximately 1,120 bp fragment encompassing part of the 3′ end of the cytochrome b gene, the tRNA-Thr and tRNA-Pro regions, and the beginning of the mitochondrial control region (D-loop). PCR reactions were performed using the Phire Animal Tissue Direct PCR Kit (Thermo Fisher Scientific, USA) in a final volume of 20 µL containing 10 µL Phire Tissue Direct Buffer, 0.20 µL Phire HS II DNA polymerase, 0.60 µL of each primer (10 µM), 2 µL genomic DNA template at 15 ng/µL, corresponding to approximately 30 ng DNA per reaction, and 6.60 µL nuclease-free water. In no-template controls, genomic DNA was replaced by nuclease-free water.

Amplifications were carried out using a Bioer LifeEco TC-96 thermal cycler (Hangzhou Bioer Technology Co. Ltd., China) under the following cycling conditions: initial denaturation at 98 °C for 5 min; 35 cycles of 98 °C for 10 s, 62.1 °C for 25 s, and 72 °C for 20 s;

followed by a final extension at 72 °C for 5 min. PCR products were verified by electrophoresis on 1.5% agarose gels using a GeneRuler 100 bp DNA Ladder (Thermo Scientific, USA).

Chromatograms were manually inspected and consensus sequences were assembled using Geneious Prime® (version 2025.1.3). For phylogenetic analysis, only the mitochondrial control region (D-loop) was retained, while conserved flanking regions corresponding to cytochrome b and tRNA genes were excluded prior to alignment. After trimming low-quality ends and removing flanking conserved regions, the final aligned D-loop fragment had a length of 866 bp.

Multiple sequence alignment was performed using the MUSCLE algorithm implemented in MEGA11 (Tamura et al., 2021). Phylogenetic relationships were inferred using the Maximum Likelihood method in MEGA11. The Tamura-Nei model (TN93) with uniform rates among sites was used for tree reconstruction. Bootstrap support values were calculated using 1,000 replicates. Gaps and missing data were treated using the "use all sites" option. The Maximum Likelihood tree was inferred using the Nearest-Neighbor-Interchange (NNI) heuristic method and visualised as a rectangular tree.

Genotyping of production- and adaptation-related nuclear genes

The selected loci represent well-characterised functional polymorphisms affecting milk composition, lipid metabolism and thermoregulation, traits potentially relevant for production performance and environmental adaptation in extensive pastoral systems.

The analysed genes included members of the casein gene cluster located on bovine chromosome 6 (*CSN1S1*, *CSN2*, *CSN1S2* and *CSN3*) (Meier et al., 2019), together with *LGB*, which is associated with milk protein composition (Soyudal et al., 2019), and additional loci associated with milk fat synthesis (*DGAT1*) (Čítek et al., 2022) and thermoregulation (*PRLR/SLICK*, corresponding to the *SLICK* locus) (Littlejohn et al., 2014). Targeted genotyping was performed using gene-specific primers described in Table 1.

Table 1 Primer sequences used for amplification of candidate nuclear genes associated with milk composition, lipid metabolism and thermoregulation

Gene ID	Primer	Sequence (5′–3′)	Amplicon size (bp)	Reference
CSN1S1	Forward	CCCATTGGCTCTGAGAACGG	247	*Mohan et al., 2021*
	Reverse	CACTGCTCCACATGTTCCTG		
CSN2	Forward	TTTCCAGGATGAACTCCAGGAT	547	*Sebastiani et al., 2020*
	Reverse	CATCAGAAGTTAAACAGGCACAGTTAG		
CSN1S2	Forward	CTTGCCATCAAAACAAACAGG	280	*Mohan et al., 2021*
	Reverse	TTTTCGTTATGGTCGCACTTC		
CSN3	Forward	CACGTCACCCACACCCACATTTATC	379	*Soyudal et al., 2019*
	Reverse	TAATTAGCCCATTTCGCCTTCTCTGT		
LGB	Forward	TGTGCTGGACACCGACTACAAAAA	247	*Soyudal et al., 2019*
	Reverse	GCTCCCGGTATATGACCACCCTCT		
	Forward	GCACCATCCTCTTCCTCAAG	413	

Gene ID	Primer	Sequence (5′–3′)	Amplicon size (bp)	Reference
DGAT1	Reverse	GGAAGCGCTTTCGGATG		*Čítek et al., 2022*
PRLR/SLICK	Forward	CCTATTTTCTGGCCAATGGA	598	*Littlejohn et al., 2014*
	Reverse	CAGCCCAACTGGAGTCTGC		

PCR reactions for nuclear gene amplification were performed in the same final volume and using the same reaction mixture as described above for mitochondrial D-loop amplification, with gene-specific primer pairs used for each selected locus. For each PCR run, no-template controls (NTC) were included in parallel to monitor potential contamination.

Thermal cycling conditions were identical to those described above for mitochondrial D-loop amplification, except that gene-specific annealing temperatures were used. An annealing temperature of 61.1 °C was applied for *CSN1S1*, *CSN2*, *CSN1S2* and *PRLR/SLICK*, whereas 62 °C was used for *CSN3*, *LGB* and *DGAT1*.

PCR products were separated by electrophoresis on 1.5% agarose gels at 130 V for 30 min. Fragment sizes were estimated using a GeneRuler 100 bp Plus DNA Ladder. Gels were stained with ethidium bromide and visualised under UV illumination. Clear PCR products corresponding to the expected fragment sizes were obtained for the reactions included in downstream sequence analysis. No amplification was detected in the NTC controls, indicating the absence of detectable contamination. PCR products were purified and subjected to bidirectional Sanger sequencing performed by Eurofins Biomi Kft. (Gödöllő, Hungary).

The analysed functional polymorphisms and their genomic positions are summarised in Table 2.

Table 2 Functional SNP polymorphisms analysed in Kuri cattle

Gene	Reference sequence (GenBank)	Chr	Position (UMD3.1)	REF	ALT	dbSNP ID	Variant consequence
CSN1S1	X59856	6	85427427	A	G	rs43703010	*Missense variant*
CSN2	X14711	6	87181619	G	T	rs43703011	*Missense variant (β-casein region)*
CSN1S2	M94327	6	85533780	C	T	rs441966828	*Missense variant*
CSN3	AY380228	6	85656736	T	C	rs43703015	*Missense variant*
LGB	X14710	11	103259232	C	T	rs109625649	*Missense variant*
DGAT1	NM_174693	14	611019	G	A	rs109234250	*Missense variant (K232A region)*

Gene	Reference sequence (GenBank)	Chr	Position (UMD3.1)	REF	ALT	dbSNP ID	Variant consequence
DGAT1	NM_174693	14	611020	A	C	rs109326954	*Missense variant (K232A region)*
PRLR/SLICK	NM_001012370	20	39099113	C	G	–	*Stop-gained variant (SLICK4, p.Tyr427*)*
PRLR/SLICK	NM_001012370	20	39099214	C	DEL	rs517047387	*Frameshift deletion (SLICK1, p.Leu462*)*
PRLR/SLICK	NM_001012370	20	39099226	C	A	rs533447499	*Stop-gained variant (SLICK3, p.Ser465*)*
PRLR/SLICK	NM_001012370	20	39099228	A	T	–	*Stop-gained variant (SLICK5, p.Lys466*)*
PRLR/SLICK	NM_001012370	20	39099267	C	T	–	*Stop-gained variant (SLICK6, p.Gln479*)*
PRLR/SLICK	NM_001012370	20	39099321	C	T	rs533447470	*Stop-gained variant (SLICK2, p.Arg497*)*

Reference gene sequences were obtained from the NCBI GenBank database. Genomic coordinates correspond to the Bos taurus genome assembly UMD3.1, and variant identifiers refer to entries in the NCBI dbSNP database where available.

Statistical analysis

Allele and genotype frequencies were calculated by direct gene counting and are presented as descriptive values only. Because of the small sample size and the unknown relatedness among sampled individuals, observed and expected heterozygosity values were not interpreted as population-level diversity estimates. Calculations were performed manually in Microsoft Excel.

Results and discussion

Mitochondrial DNA phylogeny

Phylogenetic relationships were inferred using Maximum Likelihood analysis based on 866 bp mitochondrial D-loop sequences of the analysed Kuri individuals together with

representative Bos reference sequences retrieved from GenBank (Figure 2). The resulting topology revealed mitochondrial heterogeneity among the eight Kuri samples. Several individuals formed small internal clusters characterised by short branch lengths, reflecting limited nucleotide divergence within the analysed D-loop fragment. For example, samples 7291, 7292 and 7295 grouped together, while samples 7293 and 7294 formed a separate cluster. However, bootstrap support values within these internal groupings were generally low, indicating limited phylogenetic resolution in this mitochondrial segment.

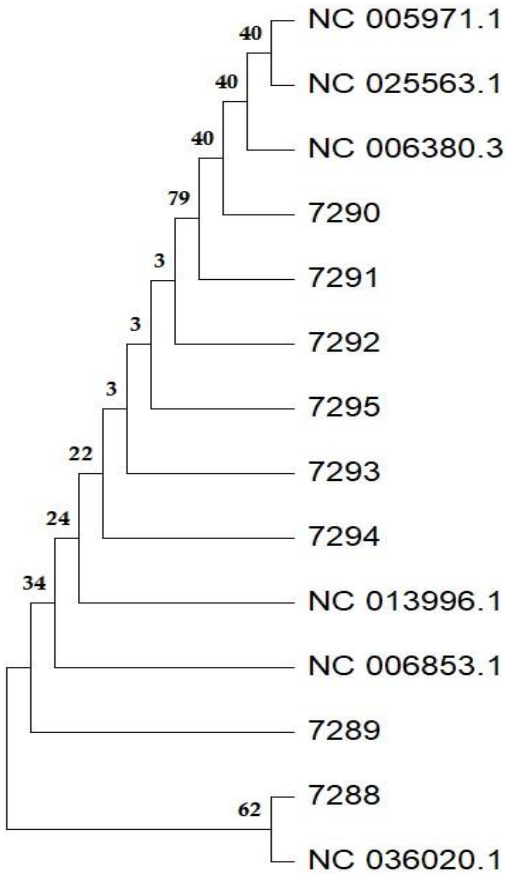

Figure 2 Maximum Likelihood tree based on 866 bp mitochondrial D-loop sequences reconstructed in MEGA11 using the Tamura-Nei model (TN93) with uniform rates among sites

Bootstrap values based on 1,000 replicates are indicated at the nodes. Reference sequences include representatives of Bos indicus (NC005971.1), Bos mutus (NC025563.1), Bos grunniens (NC006380.3), Bos primigenius (NC013996.1), Bos taurus (NC006853.1), and Bos frontalis (NC036020.1). Kuri cattle samples are indicated by their sample codes.

Some individuals occupied more isolated positions in the Maximum Likelihood tree relative to the other Kuri samples. In the present analysis, sample 7288 was placed adjacent to the *Bos frontalis* reference sequence (NC036020.1), with moderate bootstrap support, whereas sample 7290 was positioned near a group containing several *Bos* reference lineages. However, these placements should not be interpreted as evidence of taxonomic affinity or species-level relationship. Because the analysed fragment represents a relatively short portion of the hypervariable mitochondrial control region and only a limited number of

reference sequences were included, the observed topology most likely reflects local sequence similarity within the analysed D-loop fragment.

Bootstrap values were generally low at several internal nodes, which likely reflects both the limited fragment length (866 bp) and the small number of analysed individuals. Therefore, the analysis was used to describe broad phylogenetic patterns rather than to assign formal haplogroups or haplotypes. Nevertheless, the heterogeneous placement of the analysed individuals indicates that mitochondrial sequence variation is present among the examined Kuri cattle. These results suggest that mitochondrial diversity persists in this geographically restricted breed and may provide useful preliminary information for future genetic studies and conservation programmes targeting Kuri cattle.

Nuclear functional genetic variation in production- and adaptation-related loci

Sequencing of exon 7 of the ß-casein gene (*CSN2*) revealed polymorphism at several nucleotide positions compared with the reference sequence X14711. Three genotypes were observed among the analysed individuals: A1/A1 (n = 2), A1/A2 (n = 4) and A2/A2 (n = 2), resulting in equal allele frequencies (A1 = 0.50; A2 = 0.50).

Polymorphism was also detected in several additional milk protein genes. At the *CSN1S1* locus, genotype frequencies were A/A (n = 4), A/G (n = 2) and G/G (n = 2). At the *CSN1S2* locus, genotype frequencies were C/C (n = 5), C/T (n = 2) and T/T (n = 1). At the selected *CSN3* T>C polymorphism, three genotype classes were observed among the eight analysed individuals: C/C (n = 3), C/T (n = 3) and T/T (n = 2). At the *LGB* locus, genotype frequencies were C/C (n = 3), C/T (n = 2) and T/T (n = 3), resulting in equal allele frequencies (C = 0.50; T = 0.50).

Variation was also observed in the *DGAT1* gene, where individuals exhibited K/K and K/A genotypes at the p.K232A polymorphism, indicating segregation of the well-known DGAT1 K232A variant associated with milk fat synthesis. The K allele of this polymorphism has been associated with increased milk fat content, whereas the A allele is typically linked to lower milk fat levels in dairy cattle populations (Grisart et al., 2002; Winter et al., 2002).

In contrast, all six screened *PRLR/SLICK*-associated variants were homozygous wild-type in all individuals. None of the previously reported PRLR variants associated with the *SLICK* phenotype were detected in the analysed animals.

The genotype distribution of the analysed loci is summarised in Table 3.

Table 3 Genotype distribution of analysed nuclear loci in Kuri cattle (n = 8)

Gene	Genotype	Number of individuals	Frequency
CSN1S1	A/A	4	0.50
CSN1S1	A/G	2	0.25
CSN1S1	G/G	2	0.25
CSN2	A1/A1	2	0.25
CSN2	A1/A2	4	0.50
CSN2	A2/A2	2	0.25
CSN1S2	C/C	5	0.625
CSN1S2	C/T	2	0.25

Gene	Genotype	Number of individuals	Frequency
CSN1S2	T/T	1	0.125
CSN3	C/C	3	0.375
CSN3	C/T	3	0.375
CSN3	T/T	2	0.250
LGB	C/C	3	0.375
LGB	C/T	2	0.25
LGB	T/T	3	0.375
DGAT1	K/K	5	0.625
DGAT1	K/A	3	0.375
PRLR/SLICK	WT/WT at all screened variants	8	1.00

DGAT1 genotypes are shown at the protein level for the p.K232A polymorphism. CSN3 genotypes are shown for the selected biallelic rs43703015 T>C polymorphism only and were successfully scored in all eight individuals. For PRLR/SLICK, WT/WT indicates the homozygous wild-type genotype at all six screened SLICK-associated variants listed in Table 2.

Allele frequencies calculated for the analysed loci are presented in Table 4. Because of the small sample size, the unknown relatedness among sampled individuals and their origin from different herds, these values should be interpreted as descriptive results only and not as population-level estimates. Several milk protein genes showed detectable polymorphism within the analysed sample, particularly *CSN1S1*, *CSN2*, *CSN1S2*, the selected *CSN3* rs43703015 SNP and *LGB*. Variation was also observed at the *DGAT1* locus, whereas the screened *PRLR/SLICK*-associated variants were monomorphic in all individuals.

Table 4 Allele frequencies of analysed nuclear loci in Kuri cattle (n = 8)

Gene	Allele	Frequency
CSN1S1	A	0.63
CSN1S1	G	0.37
CSN2	A1	0.50
CSN2	A2	0.50
CSN1S2	C	0.75
CSN1S2	T	0.25
CSN3	C	0.56
CSN3	T	0.44
LGB	C	0.50
LGB	T	0.50
DGAT1	K	0.81
DGAT1	A	0.19
PRLR/SLICK	WT at all screened variants	1.00

Comparative interpretation in international context

The mitochondrial D-loop sequences obtained in the present study were compared with representative reference sequences of *Bos indicus, Bos taurus, Bos primigenius, Bos mutus, Bos grunniens and Bos frontalis.* The analysed Kuri individuals did not form a single uniform mitochondrial cluster, but showed heterogeneous placement relative to the reference lineages, suggesting the presence of more than one maternal lineage within the sampled animals. One individual was placed adjacent to the *Bos frontalis* reference sequence in the analysed D-loop tree, whereas other individuals occupied positions closer to taurine- and indicine-associated reference sequences. However, given the short mitochondrial fragment and the limited number of reference sequences, this pattern should be interpreted as local sequence similarity rather than evidence of taxonomic affinity. Therefore, these relationships should be interpreted cautiously and should not be considered a definitive breed-level or taxonomic assignment.

The multilocus nuclear variation observed in the analysed animals can also be compared with patterns reported in other cattle populations worldwide, particularly in African breeds. Functional polymorphisms within the casein gene cluster have been widely studied because of their influence on milk composition and technological properties (Caroli et al., 2009; Grădinaru et al., 2018).

The balanced distribution of A1 and A2 alleles at the *CSN2* locus contrasts with patterns observed in several specialised dairy breeds, where the A1 allele often predominates as a result of intensive selection for milk yield (Kamiński et al., 2007). In contrast, indigenous or locally adapted cattle populations frequently show higher frequencies of the A2 allele, which has been suggested to represent the ancestral variant of the ß-casein gene (Caroli et al., 2009). Similar patterns have been reported in several African cattle populations, where the predominance of the A2 allele reflects the persistence of taurine genetic backgrounds prior to the widespread introgression of indicine lineages (Hanotte et al., 2002; Freeman et al., 2004). The equal allele frequencies observed in the present study may therefore reflect the historically admixed taurine–zebu genomic background previously described in Kuri cattle populations of the Lake Chad Basin (Hanotte et al., 2002).

Polymorphism detected in additional milk protein genes such as *LGB* and the selected *CSN3* SNP is consistent with patterns reported in cattle populations worldwide. Variants in ß-lactoglobulin and κ-casein genes have been associated with differences in milk protein content, coagulation properties and cheese yield (Soyudal et al., 2019; Grădinaru et al., 2018). The observed variation at these loci indicates that genetic variation affecting milk composition is still present in this population. Comparable polymorphism in milk protein genes has also been reported in several indigenous African breeds, including N'Dama, Boran and Ankole cattle, which maintain considerable genetic diversity despite relatively small effective population sizes and extensive production systems (Ibeagha-Awemu et al., 2004; Rege et al., 2025).

The *DGAT1* gene plays an important role in milk fat synthesis and represents one of the most extensively studied functional polymorphisms in cattle (Čítek et al., 2022). The coexistence of K and A alleles at the *DGAT1* p.K232A locus in the analysed Kuri individuals indicates that this economically important milk-fat-associated variant is segregating in the examined animals. Previous studies have shown that the *DGAT1* K232A polymorphism occurs across both *Bos taurus* and *Bos indicus* cattle breeds, with variable allele frequencies among breeds and production types (Kaupe et al., 2004). Similar *DGAT1*-related variation has also been investigated in indigenous African cattle populations,

including White Fulani and Borgou cattle from Benin and several Ethiopian cattle populations (Houaga et al., 2018; Samuel et al., 2022).

In contrast, no polymorphism was detected among the screened *PRLR* variants associated with the *SLICK* phenotype. *SLICK*-associated mutations have been described in several tropical cattle populations and are associated with improved heat dissipation through reduced hair length (Littlejohn et al., 2014). The absence of the screened *SLICK*-associated variants in these eight individuals suggests that thermotolerance in the sampled Kuri cattle is unlikely to be mediated by these known major-effect *PRLR* mutations. Instead, environmental adaptation in this breed may involve alternative physiological mechanisms or polygenic genetic architectures, as reported in other indigenous cattle populations adapted to harsh environmental conditions (Boettcher et al., 2015).

Such adaptation likely involves a combination of physiological and behavioural mechanisms typical of cattle populations adapted to hot environments, including increased sweating capacity, enhanced skin vascularisation and improved respiratory heat dissipation (Boettcher et al., 2015; Groeneveld et al., 2010). Behavioural responses such as frequent contact with water bodies and movement between islands may further facilitate thermoregulation under the semi-aquatic pastoral conditions characteristic of the Lake Chad Basin.

Beyond their genetic characteristics, Kuri cattle represent a distinctive example of ecological adaptation among African livestock populations. The breed is closely associated with the dynamic ecosystem of the Lake Chad Basin, where animals are traditionally maintained in semi-aquatic pastoral systems characterised by seasonal flooding, island grazing and frequent contact with shallow water bodies. Historical observations indicate that Kuri cattle regularly move between islands in search of grazing areas (Epstein, 1971; Tawah et al., 1997). Such ecological conditions may have contributed to the maintenance of locally adapted genetic variation shaped by long-term environmental pressures.

The presence of polymorphism at several production-related loci suggests that Kuri cattle still harbour functional genetic diversity. Indigenous African cattle populations are known to maintain substantial adaptive genetic variation shaped by long-term environmental selection and extensive pastoral production systems (Groeneveld et al., 2010; Lenstra et al., 2014; Hanotte et al., 2002). Despite their geographically restricted distribution and increasing demographic pressure, the present results indicate that Kuri cattle retain measurable genetic variability at loci associated with production traits and environmental adaptation.

Because only a small number of individuals were analysed, allele frequency estimates should be interpreted as descriptive rather than representative of the entire breed. Nevertheless, the data provide preliminary molecular information that may support future studies investigating the genetic structure and conservation status of Kuri cattle. Analyses including larger sample sizes and genome-wide markers will be necessary to confirm the patterns observed in this preliminary dataset.

Conclusions

The present study offers an initial molecular insight into mitochondrial and functional nuclear genetic variation in Kuri cattle from the Lake Chad Basin. Analysis of the mitochondrial D-loop region revealed heterogeneous mitochondrial sequence patterns, suggesting that mitochondrial diversity is still present in this geographically restricted

population. Nuclear genotyping revealed polymorphism at multiple loci associated with milk composition and lipid metabolism, including genes of the casein cluster and *DGAT1*. The selected *CSN3* polymorphism was evaluated as a single biallelic SNP. In contrast, none of the screened *SLICK*-associated *PRLR* variants were detected, indicating that heat adaptation in the sampled Kuri cattle is unlikely to be explained by these known *PRLR* mutations. Instead, thermoregulatory adaptation in this breed may involve other physiological or polygenic mechanisms. Although the present analysis is based on a small number of individuals, the results provide baseline information on both maternal lineages and functional genetic variation in Kuri cattle. These findings may contribute to future studies aimed at understanding the genetic structure and supporting the conservation of this distinctive African cattle population under increasing environmental and demographic pressure.

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