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# Mitochondrial DNA diversity of Tanganyika Shorthorn Zebu cattle in Tanzania

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**Abstract.** Indigenous cattle of sub-Saharan Africa exhibit high phenotypic variability, which in part is due to influence of environmental factors to which they have been exposed during their evolution. Analysis of genetic diversity at molecular level is essential for making informed decisions regarding choice of potential sub-populations for breeding and conservation. The objective of this study was to analyse genetic diversity of Tanganyika Shorthorn Zebu cattle in the Lake Victoria Basin area, which harbours a large indigenous cattle population in Tanzania. A total of 25 cattle of Sukuma and Tarime strains from Misungwi and Maswa Districts were genotyped by targeting 1200bp of mtDNA D-loop. Resulting sequences were used to estimate genetic diversity indices, analyse haplotype network and construct a Maximum-Likelihood Tree. Both sub-populations revealed high genetic diversity, with few individuals (8%) showing admixture between them. TheTarime strain tended toretain more genetic diversity than the Sukuma strain; and thus recommended as a target sub-population for selective breeding and genetic conservation of Tanganyika Shorthorn Zebu cattle in the Lake Victoria Basin of Tanzania. Further population genetics studies for indigenous cattle employing highly detective haplotype analysis tools are recommended in the area to clarify if the genetically divergent haplotypes observed could be a different sub-species or strain.

**Key words:** D-loop, genetic divergence, haplotype, nucleotide

## Introduction

African continent is richly endowed with cattle genetic resource diversity. Its indigenous cattle populations are known to exhibit high performance and morphological variability (Weerasinghe *et al*., 2018). The variability is, in part, influenced by environmental factors to which the cattle populations have been exposed during their evolution (Gaitan *et al*., 2016; Dessie and Mwai, 2019). This indicates the existence of potential for selective breeding and conservation of the animal genetic resources in the respective agro-ecologies in which they evolved.

Indigenous cattle populations found in Africa are known to have originated from both *Bos taurus* and *Bos indicus* cattle, which were domesticated in Asia (Felius *et al*., 2014; Kim *et al*., 2023). Taurine cattle entered the continent earliest through Egypt and some migrated westwards to North of Africa; whereas others migrated southwards to Eastern Africa (Felius *et al*., 2014). Lake Victoria Basin area

lies just below the convergence area and represents the part of the African continent where cattle are most concentrated (Dessie and Mwai, 2018; Weerasinghe *et al*., 2018). This suggests that, from the area of convergence, most of the cattle migrated southwards into the Lake Victoria Basin. One southward cattle migration route originating from the point of convergence, which passed nearby South of Lake Victoria in Tanzania, is that of Sanga cattle which passed in Uganda and Rwanda in the West of Lake Victoria down to Southern Africa (Rewe *et al*., 2009).

Another entry corridor for cattle into Africa before the colonial era was in Somalia at the Horn of Africa (Brass, 2018). Most of the indigenous cattle populations found in areas South of Lake Victoria in Tanzania have descended from Zebu cattle which entered through the point (Williamson and Payne, 1965; Weerasinghe et al., 2018). The cattle did not head to the point of convergence in the West, but instead went southwards crossing Kenya into Tanzania, where at some locations met Sanga cattle emigrated southwards from the point of convergence (Williamson and Payne, 1965; Dessie and Mwai, 2018). Most of Sanga cattle were displaced between 1887 and 1893 during the Rinderpest epidemic, which killed around 90% of Sanga cattle and only around 30% of pure Zebu cattle (Felius *et al*., 2014). Within the Lake Victoria Basin area of Tanzania, interbreeding of Sanga cattle from West and Zebu cattle from East of Lake Victoria was also implied by Huber *et al*. (2008). Considering that the Sanga cattle is a hybrid of Longhorn and Shorthorn cattle, which entered the continent from Egypt, then indigenous cattle populations in the Lake Victoria Basin area of Tanzania are implied to carry a diverse pool of genes that enabled the cattle to adapt to various rigorous environmental and managerial conditions existing from North to Eastern Africa.

Analysis of genetic diversity at molecular level is thus important for exploring avenues for selective breeding of these livestock populations to be able to make informed decisions regarding making choices of sub-populations to breed and conserve. Various works have been done during recent decades to study genetic diversity of African cattle. From most of the studies, significant within and between breed genetic diversity was reported (Rewe *et al*., 2009; Msalya *et al*., 2017; Weerasinghe *et al*., 2018). However, there is the need for such studies to be repeated over time in the similar agro-ecological conditions to validate the results. The methodological approaches employed to analyse the diversity are changing and new ones, which tend to be more informative have been invented (Demir *et al*., 2023).

The past studies which analysed genetic diversity of indigenous cattle in the Lake zone of Tanzania used microsatellite, Random Amplified Polymorphic Deoxyribonucleic Acid and Single Nucleotide Polymorphism markers, all of which utilised nuclear DNA (Msalya *et al*., 2017). Recently, mitochondrial DNA has gained superiority over nuclear DNA as a source of markers for analysing related sub-populations, due to their conservatism and maternal inheritance (Daniel and Brenden, 2005). The objective of this study was to analyse genetic diversity of Tanganyika Shorthorn Zebu cattle in the Lake Victoria Basin area, which harbours a large indigenous cattle population in Tanzania.

## Materials and methods

### Description of the study area

The study was conducted in Misungwi District of Mwanza Region and Maswa District of Simiyu Region, both located in the Lake Victoria Basin area of Tanzania. These are among districts with the biggest number of Zebu cattle in the Lake Victoria Basin area; and in both Districts Sukuma and Tarime strains are commonly found in farmers' herds (Maswa, 2022; Misungwi, 2022).

The cattle production system in both districts is grassland-based, with agro-pastoral nature (URT, 2017). Vegetation cover is predominated by thorny trees mainly of Acacia species (Olago *et al*., 2006).

Cultivation intensity is moderate and common crops grown are rice, maize, sorghum and cotton (URT, 2017).

## Data collection

### Tissue sample collection and DNA extraction

This study followed guidelines for carrying out cattle molecular diversity studies outlined by FAO (2011), which specify a minimum sample size of 25. It used 25 Tanganyika Shorthorn Zebu (TSZ) cattle of Sukuma (n=13) and Tarime (n=12) strains, which were sampled randomly from eight villages of Misungwi and Maswa Districts. At sampling, herd histories were used to ensure sampled cattle had different lineages. Solid tissue samples were collected from the sampled cattle, by first restraining them in crush, and chopping a small flesh piece of about 40 mg at the tip of the ear, sanitised with 70% Ethanol, using a sterile surgical blade. Each flesh piece was preserved in absolute Ethanol, in eppendorf tubes; and placed in a cool box and taken to the molecular biology laboratory of the department of Zoology and Wildlife Conservation, University of Dar es salaam for storage at -20 $^{o}$C.

Genomic DNA extraction was done using Zymo Quick-DNA™ Miniprep-Plus kit (manufactured by Zymo Research Corporation, USA); following manufacture's protocol for solid tissue samples (Suzuki *et al*., 1993). Subsequently, the tissues samples were macerated, subjected to 200 µl (10 µl Proteinase K (20 mg $l^{-1}$), 95 µl Solid Tissue Buffer and 95 µl nuclease free water); and incubated overnight at 56$^{o}$ C. Twelve extracted DNA samples (6 of Sukuma and other 6 of Tarime cattle) were randomly selected and used to confirm DNA by gel electrophoresis on 1% Agarose gel. Quality of the gel was examined visually by clarity at the initial and end point of the ladder. The extracted DNA was stored at -20$^{o}$ C for conventional PCR.

### DNA amplification and sequencing

About 1200bp of mtDNA D-loop region was amplified using Suzuki *et al*. (1993) primers.Conventional PCR was performed using sequences 5´-TAGTGCTAATACCAACGGCC-3´, and 5´-AGGCATTTTCAGTGCCTTGC-3´ for forward and reverse reactions, respectively (Suzuki *et al*., 1993). The reaction used a total volume of 25 µl containing 5 µL 10X MyTaq reaction buffer (Bioline), 1 µl of 10 mM forward and Reverse primers, 0.3 µl MyTaq DNA polymerase (Bioline), 1 µl genomic DNA, and 16.7 µl of nuclease free water. PCR was carried out in a MyGene™ Series Peltier Thermocycler (model MG96G)at 94 $^{O}$C for 6 minutes (initial denaturation)followed by 35 cycles of denaturation at 94 $^{O}$C for 40 seconds, annealing at 56 $^{O}$C for 1 minute, and extension at 72 $^{O}$C for 1 minute. Final extension was carried at 72 $^{O}$C for 10 minutes. The PCR products were confirmed through gel-electrophoresis using the 12 randomly selected samples (6 each for Sukuma and Tarime cattle specimens) in 1% Ethidium bromide stained gel under 10 kb Hyper™ ladder. The PCR products were purified by exo-SAP and bidirectionally Sanger sequenced commercially at Psomagen sequencing centre (New York, USA) using same primers.

### Data analysis

Raw sequences of the Zebu cattle were manually edited using BioEdit software (Hall, 1999). This involved quality filtering to minimise laboratory errors. Consensus sequences were blasted against reference sequences in NCBI (www.blast.ncbi.nlm.nih.gov), at query cover and percentage identity of 97-100. Genetic diversity was assessed by estimating haplotype diversity ($H_d$), nucleotide diversity ($\pi$), number of haplotypes (h), number of polymorphic sites (NPS) and average number of nucleotide differences (k), using DnaSP with pre-set options as described by Rozas *et al*. (2017). The clustering

pattern of sequences was determined by plotting a Templeton Crandall and Sing (TCS) haplotype network, in PopART 1.7 software (Clement *et al*., 2002) and construction of Maximum-Likelihood tree using the Jukes–Cantor approach employing 1000 Bootstrap replicates in MEGA 11 software (Tamura *et al*., 2021).

## Results

### NCBI Nucleotide Blast Results

In nucleotide blast all the Sukuma cattle, except one displayed complete query coverage (100%). On the other hand, all the Tarime cattle displayed incomplete, but also very high query coverage (99%). Hence, Sukuma cattle sequences were remarked to exhibit higher alignment to data bases than Tarime cattle sequences. However, identity in the aligning sequences was very high and similar among the cattle of both strains. Twenty three of the 25 cattle sequences showed percentage identity above 99% corresponding to *Bos taurus* cattle. This indicated high alignment of the analysed Zebu cattle DNA sequences to those of *Bos taurus* cattle in the genbank.

### Genetic diversity

The genetic diversity analysis of the Zebu cattle showed the existence of high haplotype diversity (Hd), moderately high nucleotide diversity ($\pi$), and moderate numbers of haplotypes (h), polymorphic sites (NPS) and nucleotide differences (k) (Table 1). On comparing the two strains, Tarime cattle demonstrated more genetic diversity than Sukuma cattle, with regard to Hd, $\pi$,NPS and k. Number of haplotypes observed for Sukuma (13) and Tarime (12) cattle were similar.

**Table 1.** Genetic diversity indices for the study Zebu strains in the study areas in Tanzania

| Strain | $H_d$ | $\pi$ | h | NPS | k |
|---|---|---|---|---|---|
| Sukuma | 0.857 | 0.00368 | 13 | 21 | 3.868 |
| Tarime | 0.916 | 0.00606 | 12 | 33 | 6.379 |

$H_d$ = haplotype diversity, $\pi$ = nucleotide diversity, h = number of haplotypes, NPS = number of polymorphic sites, k = average number of nucleotide differences

### Haplotype Network

Analysis of the 25 mtDNA D-loop cattle sequences produced 24 haplotypes, 23 of which were singleton and one was shared (BMISTm0630) (Fig. 1). Most of the haplotypes in both of the strains were separated by one or two mutational steps, typical of conspecifics. Only haplotype BMASTm1137 for Tarime and haplotypes BMASSk06266 and BMISSk0628 for Sukuma strains showed remarkable genetic diversity.

### Phylogenetic relationship

The Maximum Likelihood tree (Fig. 2) rooted by *Bos mutus* (OM869349.1) and *Bos indicus* (AY126697.1), resolved the 25 TSZ cattle sequences into two main clusters commensurate to the TCS haplotype network in Figure 1. The most phylogenetically distant cluster consisted of one specimen of Tarime strain (BMASTm1137), which formed a well supported clade implying that it could be a distinct species or subspecies of cattle. The second cluster with 24 specimens resolved into a distinct clade, supported by a bootstrap of 85%. The clade branched into several subclades, supported by bootstrap values of at least 50%; importantly, the clade consisted of a mixture of TSZ strains (Tarime and Sukuma).

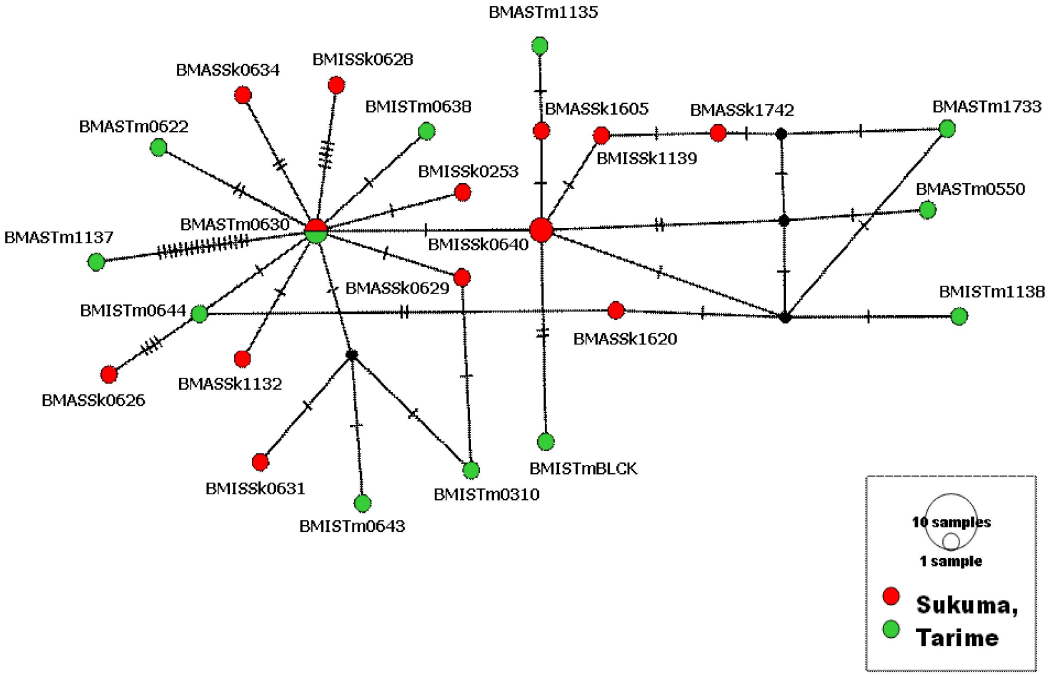


**Figure 1.** Haplotype Network for the study strains of Tanganyika Shorthorn Zebu, constructed from 1200bp nucleotide sequences from 25 cattle specimens. Each round represents a haplotype; while a single hatch mark represents a single mutational steps. A shared haplotype consists of two shared colours in one circle. Red haplotypes represent the Sukuma strain; while the haplotypes coloured green represent the Tarime strain.

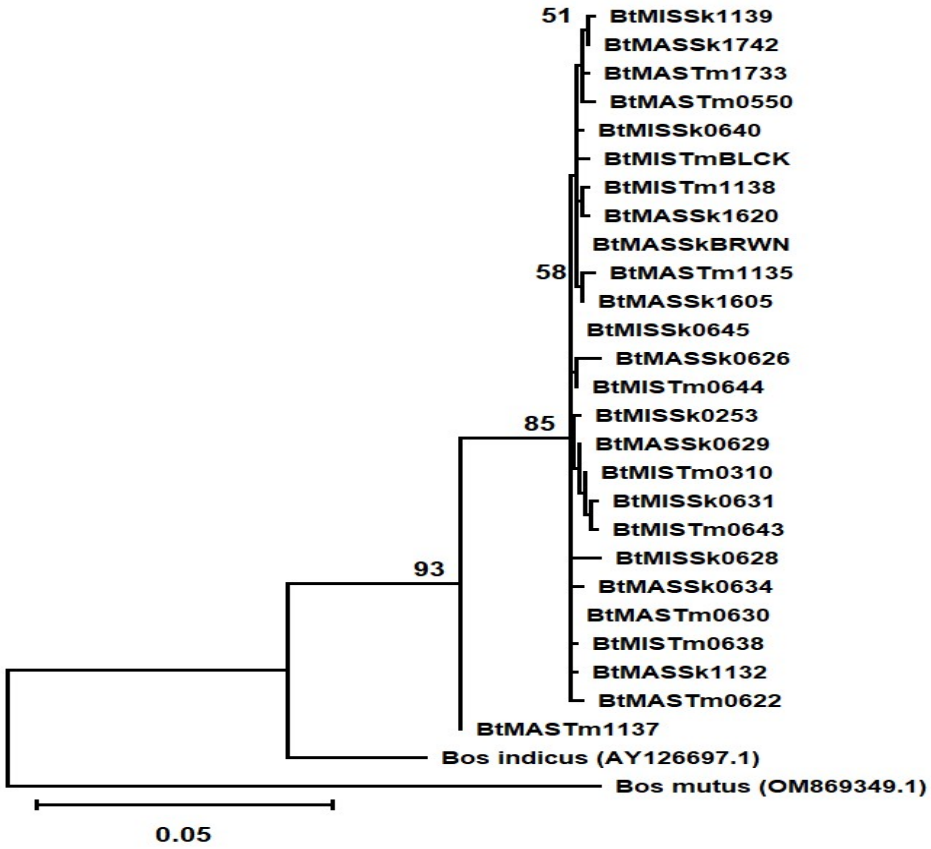


**Figure 2.** Maximum-Likelihood tree of the study Zebu strains. Scale represents the mean number of nucleotide substitutions per site on respective branch.

## Discussion

This study assessed genetic diversity of Zebu cattle of Sukuma and Tarime strains (Table 1). Haplotype diversity observed was high but slightly lower than those reported by Mauki *et al*. (2021) (Hd= 0.985) and Olivieri *et al*. (2015) (Hd=1) for Nigerian and Egyptian cattle, respectively. The high haplotype diversity implied higher maternal hereditary variance in Tarime than Sukuma strain. The variations in haplotype diversity signify differential influence of cattle populations from migratory admixing which is high in the Lake Victoria basin than other African continent regions. Sukuma are also closer than Tarime cattle to the cattle routes convergence point, a location where admixture is highest.

The estimated nucleotide diversity values in the study were moderately high for both of the studied strains. Due to their higher nucleotide diversity, Tarime cattle were implied to embrace higher hereditary variance than Sukuma cattle. The estimated values for nucleotide diversity of the Zebu cattle in this study are within the range 0.001-0.004, which has been reported for East African Zebu and Sanga cattle (Zegeye *et al*., 2023; Tijjani *et al*,. 2024). This suggests common origin and similar subjection to genetic erosion among East African Zebu cattle populations.

The values for Number of polymorphic sites (NPS) for the studied Zebu cattle strains were moderate, but lying below the mean (58) reported by Mauki *et al*. (2021) for East and Northern Africa Zebu cattle. This also signifies the existence of genetic dilution in the studied Zebu cattle sub-populations. Disaggregation of the results portrayed superiority of Tarime over Sukuma strain, similarly to Hd and $\pi$. Numberof nucleotide differences (k) was also moderate but lied slightly above the average (3.46) reported for East African Zebu (Mauki *et al*., 2021). The superiority of Tarime strain over Sukuma in genetic diversity was found to be most profound with regard to k because the index value estimation is independent of the number of sampled sequences, which could have posed a limitation in other indices ($\pi$, Hd and NPS) (Nei and Tajima,1981).

Numbers of haplotypes were moderate and comparable between the strains, suggesting common or closely related lineage in evolution of the two Zebu cattle sub-populations studied (Nei and Tajima, 1981). The numbers of haplotypes observed were slightly lower than those reported in other East African Zebu breeds, which also suggests the study location to be representing a spot of high cattle admixture (Tarekegn *et al*., 2018; Mauki *et al*., 2021). This finding calls upon further investigation based on high sensitivity haplotype analysis tools for the Zebu cattle subpopulations.

Haplotype network analysis, likewise displayed existence of high genetic diversity demonstrated by high number of haplotypes (24) that were almost equal to the number of individual cattle sampled (25) (Fig. 1). Additionally, the haplotype network revealed genetic admixture between the two strains; with the exception of three haplotypes that displayed remarkable genetic divergence. The remaining haplotypes were hardly separated by two mutational steps typical of conspecifics. This implies that the two strains belong to the same species that are coherently connected by gene flow. This could be attributed by the fact that livestock keepers in the study area coherently reared the two strains and cattle from different herds often came converged during grazing and at water points; making it possible for different cattle strains to interbreed (URT, 2017). Nevertheless, the three haplotypes [Sukuma (2) and Tarime (1)] that exhibited significant genetic divergence could be separate species or subspecies. Genetic divergence has been observed in many African indigenous cattle breeds, which in part reflects complex gene flows occurred (Hanotte *et al*., 2002; Gautier *et al*., 2009).

The Maximum-Likelihood tree phylogenetically resolved the 25 TSZ cattle sequences into two clusters (Fig. 2). The first cluster consisted of a genetically distant Tarime strain specimen. Evidently, the same specimen was separated from other haplotypes by 18 mutational steps in the haplotype network; indicating genetic introgression (Hanotte *et al*., 2002). Therefore, there is the need for further molecular diversity investigation to elucidate such genetic divergence. The rest of Sukuma and Tarime cattle

were in the second cluster of the Maximum-Likelihood tree, suggesting the two strains were conspecifics (Gregory, 2008).

The study findings generally indicated that Tarime strain was genetically more diverse than Sukuma strain. The relatively low genetic diversity in Sukuma cattle represents genetic dilution consequent to introgression of Sanga cattle (Rewe *et al*., 2009; Sharma *et al*., 2015). The high genetic diversity of Tarime cattle implied for large genetic distance between individuals of the subpopulation, which in-turn indicates high capability to adapt and cope with ecological changes than Sukuma cattle. Consequently, basing on genetic diversity grounds, Tarime cattle should be considered as a focal sub-population for selective breeding and genetic conservation of TSZ cattle breed in the Lake Victoria Basin area. A unique opportunity for selective breeding and in situ conservation of Tarime strain, exists in Ukerewe Island due to the natural restriction of cattle immigration and herds intermixing (Chasama and Chang'a, 2023). Hence, policy makers and other stakeholders should advocate for the development of selection schemes and conservation programme for the TSZ cattle subpopulation in Ukerewe District.

## Conclusion

There exists high mitochondrial DNA diversity in Sukuma and Tarime cattle, which are the major Tanganyika Shorthorn Zebu cattle sub-populations found in the Lake Victoria Basin area of Tanzania. Genetic divergence and admixture between the strains also exist. Tarime strain tended to retain more genetic diversity than Sukuma cattle and hence is recommended as a target sub-population for selective breeding and genetic conservation of Tanganyika Shorthorn Zebu cattle in the Lake Victoria Basin area. Further studies employing high detective analytical tools like haplotype plot in excel (HAPPE) are recommended to investigate if the genetically divergent haplotypes observed in the present study could be a different sub-species or strain.

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