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Phylogenetic Relationships, Genetic Diversity, and Neutrality Tests of Nigerian Cattle Populations in Taraba State

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Abstract— *The research investigated the genetic diversity and genetic neutrality of cattle populations in Taraba State, Nigeria. The study analyzed 100 reference populations, and 28 blood samples were used for mitochondrial DNA sequencing using Flinders Technology Associates (FTA) paper, which covered five locations (Iware, Wukari, Donga, Gembu, and Jalingo) and five breeds (Bokoloji, Muturu, Red Bororo, White Fulani, Adamawa Gudali). The research utilized Tajima's Neutrality Test, Tajima's Relative Rate Clock Test, and phylogenetic analysis to determine patterns of molecular evolution and population structure. The analysis of location-based neutrality showed that all populations tested positive for Tajima's D, with Iware recording 2.73 D value, Wukari showing 3.33 D value, Donga presenting 1.99 D value, and Gembu achieving 4.04 D value. The nucleotide diversity (π) measured between 0.5759 in Donga and 0.6781 in Gembu, indicating moderate to high genetic variability, whereas Wukari and Gembu displayed the most segregating sites with S = 778 and 779. The findings demonstrate evolution that deviates from neutral patterns due to three factors: balancing selection, population subdivision, and historical demographic patterns. The breed-based analysis produced positive Tajima's D results, reaching peak values in White Fulani (D = 4.49) and Red Bororo (D = 4.05), with both breeds showing high nucleotide diversity (π = 0.6535 and 0.6989, respectively). The Bokoloji (D = 1.25) and Muturu (D = 1.00) results showed reduced polymorphism levels, with S = 562 and 512. The Tajima's Clock Test results showed that evolutionary rates differed significantly between study locations. Iware showed the highest number of identical sites (202) and very few divergent sites (6), but a pronounced imbalance in unique substitutions, specially in sequence A (536). Wukari, Donga, and Gembu showed more divergent sites, with their total counts reaching 244, 229, and 193 respectively, while their unique differences among sequences appeared to be distributed more evenly, which proved the molecular clock predictions to be less accurate. The analysis of phylogenetic relationships demonstrated that different breeds of cattle from different regions showed shared ancestry together with genetic mixing from different populations. The research results demonstrate that Taraba State cattle populations possess high genetic diversity together with non-neutral evolutionary patterns and different rates of evolutionary change, which affect both conservation efforts and breeding programmes.*

Keywords— Genetic diversity, Tajima's neutrality test, Cattle breeds, Phylogenetic analysis.

I. INTRODUCTION

Cattle (*Bos taurus* and *Bos indicus*) represent one of the most economically and culturally important livestock species globally, delivering essential resources that include meat, milk, hides, and draft power while functioning as vital components for both agricultural operations and rural community development (Mwai et al., 2021; Talenti et al., 2022). The cattle population in Nigeria serves as an essential resource for both food security and economic development while shaping the sociocultural traditions of northern communities who have practiced pastoralism for thousands of years (Sikiru et al., 2022). The country contains multiple native cattle breeds that developed through adverse environmental conditions, which include tropical weather patterns, transmission of trypanosomiasis and tick-borne illnesses, and the two different livestock management approaches of nomadic herding and stationary agriculture (Nwachukwu et al., 2022). The genetic structure, evolutionary links, and population history of these groups need to be examined because this knowledge helps develop

effective conservation approaches which create breeding systems that maintain production gains and safeguard essential adaptive genetic resources (Xu et al., 2025).

The evolutionary history of African cattle displays multiple complicated patterns that resulted from domestication, animal movement, and breeding between two different cattle types—humpless taurine cattle and humped zebu cattle—which began to separate from each other 150,000 to 500,000 years ago (Bonfiglio et al., 2024). Genetic and archaeological data show that taurine cattle originated in the Near East about 10,000 years ago and then spread to Africa, while zebu cattle developed from their wild ancestors in the Indus Valley about 8,000 years ago and spread throughout Africa (Pitt et al., 2022). The phenotypic diversity of contemporary African cattle results from their ability to adapt to different agro-ecological zones combined with their genetic heritage from multiple ancestral groups, which produce different taurine and indicine ancestry patterns throughout their diverse populations (Kim et al., 2023). Whole genome analysis shows that indigenous African cattle developed unique genetic characteristics through natural selection which enabled them to withstand environmental stresses, combat diseases, and survive high temperatures, thus making them key genetic resources for maintaining sustainable livestock production in tropical environments (Freitas et al., 2021; Kambal et al., 2023).

Recent genomic studies show that zebu cattle began entering African cattle herds during two major migration windows between 750 and 1,050 years ago. Research shows that African populations have strong genetic links to zebu through autosomal and Y-chromosomal zebu ancestry, while their mitochondrial DNA studies only show taurine maternal lineages, proving that male zebu ancestry entered Africa through the importation of zebu bulls from South Asia during the last three thousand years (Ward et al., 2022). This specific mitonuclear discordance pattern establishes evidence for mitonuclear coadaptation, because natural selection protects taurine mitochondrial genomes in African environments while populations maintain high levels of nuclear zebu ancestry (Kwon et al., 2022). African cattle populations today show the highest genetic diversity among all domestic livestock, with heterozygosity values between 0.30 and 0.37 and nucleotide diversity values exceeding those of European commercial breeds, because their populations maintain large effective sizes, experience minimal bottleneck events, and have continuous gene flow between groups (Freitas et al., 2021; Xu et al., 2025).

Nigeria holds the position of having the second largest cattle population in Africa, with more than 20 million cattle spread throughout its various agricultural regions (Sikiru et al., 2022). The country showcases its native cattle breeds through zebu-type breeds which include White Fulani, Red Bororo, Sokoto Gudali, Adamawa Gudali, Rahaji, and Wadara, and taurine breeds which consist of Muturu, N'Dama, and Keteku, and hybrid groups which display mixed traits (Mauki et al., 2022). Zebu cattle dominate the northern Nigerian Sahelo-Sudanian regions through extensive pastoral and transhumance practices which Fulani herders employ (Tijjani et al., 2022), while southern and Middle Belt regions house taurine breeds that benefit from their natural trypanotolerance to combat tsetse fly (*Glossina* spp.) transmitted trypanosomiasis (Nwachukwu et al., 2022). The northeastern Nigerian state of Taraba functions as an essential cattle genetic resource area because it contains multiple herds which include major genetic samples from the Mambilla Plateau, featuring its high-altitude grasslands and special weather patterns. Taraba State emerged as a key research destination for Nigerian cattle genetics studies because recent genomic research discovered 44 cattle samples from this region (Mauki et al., 2022). The combination of different environmental conditions throughout the state leads to multiple selection forces which result in different genetic patterns and adaptive abilities for regional cattle breeds that match the adaptations found in Ethiopian highland cattle (Terefe et al., 2022; Zegeye et al., 2022).

Researchers have studied the hypervariable displacement loop (D-loop) region of mitochondrial DNA through molecular analysis to study maternal ancestry, evolutionary relationships, and historical population movements among global cattle breeds (Dorji et al., 2022; Sällman Almén et al., 2022). The D-loop region exhibits high mutation rates and lacks recombination, thus researchers use it for tracing matrilineal descent and studying population distribution patterns (Demir et al., 2023). Researchers using mitochondrial DNA to conduct phylogenetic studies found distinct taurine cattle haplogroups (T, T1, T2, T3, T4, T5, P, Q, R) and zebu cattle haplogroups (I1, I2), which showed specific geographic patterns of distribution (Bonfiglio et al., 2024). African cattle studies showed that African taurine populations had a dominant T1 haplogroup distribution pattern, which indicates that strong maternal founder effects occurred during the colonization process of Africa (Pitt et al., 2022). Researchers have made recent advances in mitochondrial genomics through the development of whole mitochondrial genome sequencing, which enables better phylogenetic analysis and more precise determination of evolutionary timelines and population characteristics (Dorji et al., 2022; Sällman Almén et al., 2022). The mtDNA research of Nigerian cattle shows that the population has high haplotype diversity, because 80% of the population carries distinct haplotypes resulting from ongoing gene flow between groups (Adeola et al., 2021). Neutrality tests provide powerful

statistical frameworks for detecting departures from neutral evolution and identifying genomic regions subjected to natural or artificial selection.

The complete molecular analysis of Taraba State Nigerian cattle breeds remains unfinished because their economic value and unique genetic traits require further examination, which includes using genetic neutrality assessments to find specific genomic regions that show signs of selection (Sikiru et al., 2022; Mauki et al., 2022). This study will investigate three research areas by studying Taraba State Nigerian cattle populations through mitochondrial D-loop sequence analysis, which will provide haplotype data, establish phylogenetic links, reveal population genetic distribution, and show how evolution has deviated from neutral patterns to help create research-backed conservation methods for indigenous genetic resources which will enhance breeding efficiency and climate adaptability (Mwai et al., 2021; Xu et al., 2025).

II. MATERIALS AND METHODS

2.1 Study Area and Sample Collection:

Taraba State exists within northeastern Nigeria, extending between 6°25' N and 9°30' N and between 9°30' E and 11°45' E, covering 54,473 square kilometers. The state contains various landforms which include lowland Guinea savanna and the Mambilla Plateau, which reaches elevations above 1,600 meters to become one of West Africa's highest plateaus. The region experiences a tropical wet and dry climate which produces annual temperatures between 18°C and 35°C and yearly precipitation totals between 1,000 mm and 1,500 mm (Mauki et al., 2022).

2.2 Experimental Animals and Sample Collection:

The study used a reference population of 100 cattle, from which 28 blood samples were used for mitochondrial DNA sequencing. Blood samples were collected using Flinders Technology Associates (FTA) paper. The study used five breeds of cattle: Bokoloji, Muturu, Red Bororo, White Fulani, and Adamawa Gudali. Blood samples (5 mL) were collected from the jugular vein of apparently healthy adult cattle (>2 years old) using Flinders Technology Associates (FTA) paper. Phenotypic characteristics and herd owner information were used to identify the sampled animals. The Animal Ethics Committee of the Department of Animal Science, Federal University Wukari, Nigeria granted ethical approval for this research study, which used standard veterinary procedures after obtaining informed consent from cattle owners.

2.3 DNA Extraction:

The extraction of genomic DNA from whole blood samples was performed using a commercial DNA extraction kit which followed the manufacturer's instructions with slight adjustments that were developed specifically for cattle blood samples. The process required 200 µL of blood to be combined with 20 µL of proteinase K and 200 µL of lysis buffer (Buffer AL), which underwent incubation at 56°C for 10 minutes to achieve full cell destruction. The mixture was centrifuged at 8,000 rpm for 1 minute after the addition of 200 µL of absolute ethanol to the spin column. The column underwent two wash cycles with wash buffers (AW1 and AW2) before DNA was extracted using 100 µL of 70°C preheated elution buffer (Buffer AE). The researchers used a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, USA) to evaluate DNA concentration and purity, which showed A260/A280 ratios between 1.8 and 2.0 during the assessment of suitable samples for downstream applications. The researchers confirmed DNA integrity through the use of 1% agarose gel electrophoresis which had been stained with ethidium bromide. The researchers kept extracted DNA samples in storage at -20°C until they conducted PCR amplification.

2.4 PCR Amplification of mtDNA D-loop Region:

The mitochondrial DNA D-loop hypervariable region was amplified using universal bovine-specific primers designed to amplify an approximately 600–900 bp fragment. The primer sequences used were: Forward primer (BovDL-F): 5'-CCACTATCAGCACCCAAAGC-3' and Reverse primer (BovDL-R): 5'-GCGGGTTGCTGGTTTCACG-3' (Demir et al., 2023). PCR amplification was performed in a 25 µL reaction volume containing 12.5 µL of 2× DreamTaq Green PCR Master Mix (Thermo Scientific), 1.0 µL (10 µM) of each primer, 2.0 µL of template DNA (approximately 50–100 ng), and 8.5 µL of nuclease-free water. Amplifications were carried out in a thermal cycler (Applied Biosystems Veriti, USA) using the following cycling conditions: initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 45 seconds, and extension at 72°C for 1 minute; followed by a final extension at 72°C for 10 minutes (Dorji et al., 2022; Demir et al., 2023). PCR products were visualized on 1.5% agarose gel stained with GelRed and documented using a gel documentation system. Amplicons showing clear single bands of expected size were purified using the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's instructions.

2.5 DNA Sequencing and Sequence Editing:

Purified PCR products were sequenced bidirectionally (forward and reverse) using the Sanger sequencing method on an ABI 3730xl automated sequencer (Applied Biosystems). Sequencing reactions were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with the same primers used for PCR amplification. Raw sequence chromatograms were visually inspected for quality using Chromas v2.6.6 software (Technelysium Pty Ltd, Australia). Forward and reverse sequences were aligned and edited manually to generate consensus sequences using BioEdit v7.2.5 (Hall, 1999). Ambiguous bases and poor-quality regions at sequence terminals were trimmed, and sequences were aligned to the bovine mitochondrial DNA reference sequence (GenBank accession number V00654) to verify correct amplification of the D-loop region. Final consensus sequences were trimmed to uniform length to facilitate comparative analysis.

2.6 Sequence Alignment and Haplotype Identification:

Multiple sequence alignment of all 28 D-loop sequences was performed using the MUSCLE (Multiple Sequence Comparison by Log-Expectation) algorithm implemented in MEGA11 software (Molecular Evolutionary Genetics Analysis version 11) (Tamura et al., 2021). The MUSCLE algorithm was chosen due to its superior accuracy and speed for aligning moderately sized datasets compared to ClustalW (Edgar, 2004). Alignment parameters were set to default values with gap opening penalty of -2.9 and gap extension penalty of 0. The alignment was manually inspected and refined where necessary to ensure correct positioning of indels and to remove ambiguous alignment regions. Unique haplotypes were identified using DnaSP v6.12.03 (DNA Sequence Polymorphism) software (Rozas et al., 2017), which collapses identical sequences into single haplotypes and assigns haplotype designations.

2.7 Phylogenetic Analysis:

The phylogeny was inferred using the Maximum Likelihood method and Tamura-Nei (1993) model of nucleotide substitutions, and the tree with the highest log likelihood (-20,110.51) is shown. The initial tree for the heuristic search was selected by choosing the tree with the superior log-likelihood between a Neighbor-Joining (NJ) tree and a Maximum Parsimony (MP) tree. The NJ tree was generated using a matrix of pairwise distances computed using the Tamura-Nei (1993) model. The MP tree had the shortest length among 10 MP tree searches; each performed with a randomly generated starting tree. The analytical procedure encompassed 24 coding nucleotide sequences using 1st, 2nd, 3rd, and non-coding positions, with 790 positions in the final dataset. Evolutionary analyses were conducted in MEGA12 utilizing up to 4 parallel computing threads.

2.8 Tajima's Test for Neutrality:

Tajima's D (Tajima, 1989) compares the average number of pairwise nucleotide differences (π) with the number of segregating sites (S). Under neutral evolution, Tajima's D $\approx$ 0. Significantly negative values ($D < 0$, $P < 0.05$) indicate an excess of rare alleles consistent with population expansion, purifying selection, or selective sweeps, while significantly positive values ($D > 0$, $P < 0.05$) suggest balancing selection or population contraction (Yurchenko et al., 2023). Fu and Li's D and F* (Fu and Li, 1993) compare the number of singleton mutations with the total number of mutations (D*) or with the average number of pairwise differences (F*). Significantly negative values indicate departures from neutrality consistent with population expansion or positive selection.

III. RESULTS AND DISCUSSION

3.1 Phylogenetic Analysis:

The results of phylogenetic analysis are displayed in Figure 1. The phylogenetic tree establishes genetic relationships among different indigenous Nigerian cattle breeds through its analysis of nucleotide sequence data. The branch lengths in the study show genetic distances through their measurement system, which uses shorter branches to indicate closer genetic relationships and longer branches to show greater evolutionary divergence (Kumar et al., 2022; Stecher et al., 2020). This phylogenetic approach enables the reconstruction of evolutionary histories and assessment of genetic differentiation among cattle populations, which is essential for conservation genetics and breeding program design (Weldenegker et al., 2024; Gobena et al., 2024).

The analysis included individuals from several indigenous Nigerian cattle breeds: White Fulani, a widely distributed zebu breed known for milk production and heat tolerance; Red Bororo, a zebu breed valued for meat production and pastoral adaptability; Muturu, an endangered West African shorthorn taurine breed with trypanotolerance; Bokoloji, a lesser-known

indigenous breed; and Adamawa Gudali, a large-framed zebu breed adapted to the Adamawa highlands. The breed name and location code are included in each individual label, which uses J to represent Jalingo, G to represent Gembu, W to represent Wukari, D to represent Donga, and I to represent Iware, along with an individual identifier and sometimes sex designation where M indicates male and F indicates female. The sampling strategy uses multiple locations to assess inter-breed genetic diversity and intra-breed genetic diversity, which is essential for understanding population structure in extensively managed pastoral systems (Kim et al., 2023; Upadhyay et al., 2021).

The phylogenetic tree displays multiple genetic patterning methods which enable researchers to study how Nigerian cattle breeds evolved their relations to one another. The tree shows that members from the same breed tend to cluster together, because their genetic links remain strong even when they travel to different locations. The study results demonstrate that genetic structure depends on breed identity, because Nigerian cattle breeds show distinct genetic patterns which continue to exist after interbreeding (Tijjani et al., 2021; Weldenegker et al., 2024). White Fulani individuals create the largest genetic group, which shows significant subgroups that display high genetic diversity within their breed. This breed shows genetic variation which stems from its distribution throughout Nigeria and the Sahel area because of its large breeding population and extensive movement of herders (Kim et al., 2023; Bahbahani et al., 2021). Research into population genomics has shown that Fulani zebu cattle possess mosaic genomes which result from historical breeding between indicine and taurine cattle breeds during different time periods (Kim et al., 2023; Gobena et al., 2024).

Red Bororo individuals form a distinct cluster separate from White Fulani, despite both being classified as zebu breeds. These two pastoral populations show genetic differences because they experienced separate reproductive isolation periods and faced different selection pressures (Smetko et al., 2021). Red Bororo cattle display breed-specific selection signatures which scientists discovered through genome-wide studies that link to their thermotolerance, disease resistance, and morphological traits (Zhou et al., 2023; Porto-Neto et al., 2022). The Muturu samples form a highly divergent branch, which proves their identity as West African shorthorn taurine cattle (*Bos taurus*) with only slight zebu ancestry. The genetic distance between these two groups represents the historical separation of taurine and indicine cattle lineages which happened about 250,000 years ago (Tijjani et al., 2021; Kim et al., 2023). The Muturu population maintains unique trypanotolerance and humid tropical adaptation alleles which serve as essential genetic assets, although the population faces endangered status according to recent genomic conservation research (Tijjani et al., 2021; Barbato et al., 2022). The Bokoloji samples create a small distinct cluster, which indicates that they have different genetic characteristics compared to more commonly studied breeds. The study must proceed with caution because it has a limited sample size of two individuals. The researchers need to conduct expanded sampling because it is necessary to establish the genetic position of this breed which exists within the broad range of Nigerian cattle diversity (Kardos et al., 2021).

The breed clusters display geographic patterns which become most evident through the analysis of White Fulani samples from different regions. This pattern arises from two factors which include localized adaptation to different agro-ecological zones and management practices that prevent gene flow and founder effects which exist in particular herding communities (Ginja et al., 2023; Weldenegker et al., 2024). Researchers found through population genomic research on African cattle that environmental factors and ethnic breeding methods created distinct genetic patterns in different populations (Kim et al., 2023; Upadhyay et al., 2021). The phylogenetic structure that this analysis reveals establishes two key effects which impact both scientific research on evolution and the development of effective conservation methods. Genetic testing showed clear distinctions between the five breeds which include White Fulani, Red Bororo, Muturu, Bokoloji, and Adamawa Gudali. The endangered breed Muturu displays special significance because it possesses exclusive taurine ancestry and special adaptive features which zebu breeds do not possess (Tijjani et al., 2021; Gobena et al., 2024). The subclustering observed within White Fulani and Red Bororo suggests maintenance of substantial genetic variation, which is favorable for adaptive potential and breeding programs. High within-breed diversity protects populations from inbreeding depression while supplying breeders with essential genetic material to select for their needs during times of environmental shifts (Kardos et al., 2021; Weldenegker et al., 2024). The tree displays breed-level differences, but some individuals show intermediate placements because of their historical or current blending with other groups. West African cattle already display through their genome-wide SNP studies which demonstrate their expected mutation patterns.

The current phylogenetic study which depends on restricted sequence information shows useful results about breed connections, although complete genome analysis with thousands of SNPs and whole-genome sequencing will provide better results for identifying minor population patterns, modern hybridization events, and selection signatures (Weldenegker et al., 2024; Gobena et al., 2024). The best method to fully assess Nigerian cattle genetic resources requires researchers to combine phylogenetic data with population genomic data and phenotypic data (Kim et al., 2023; Ginja et al., 2023).

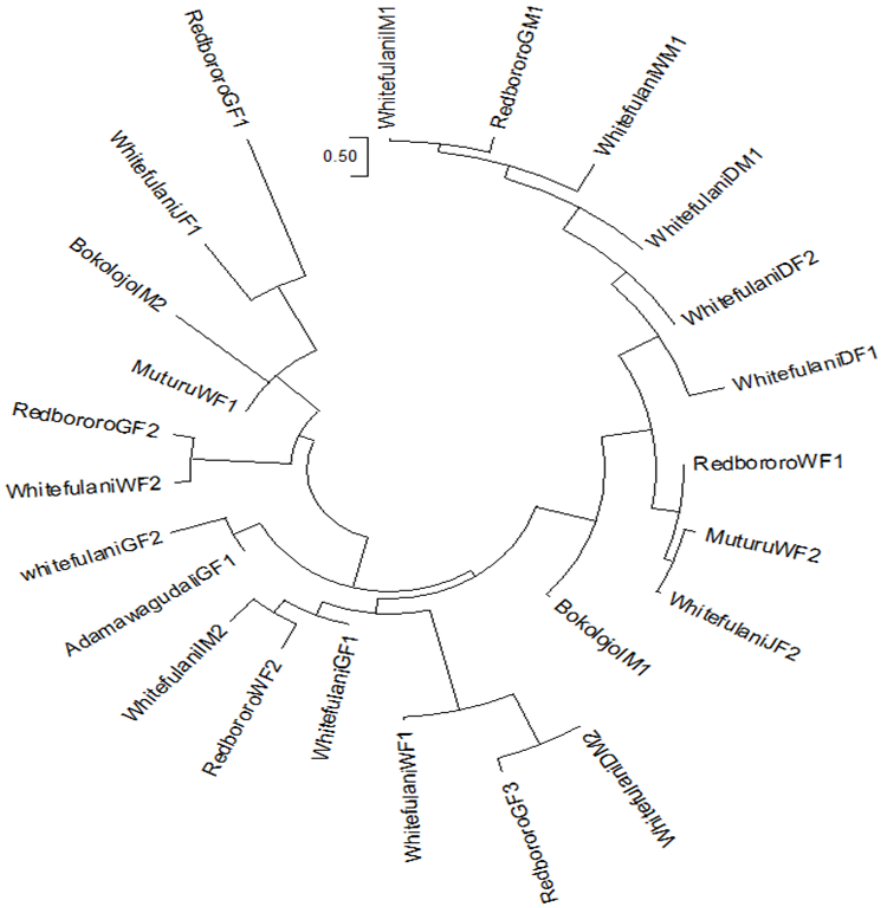

FIGURE 1: Evolutionary analysis of cattle in Taraba State

3.2 Tajima's Neutrality Test Results:

The results of Tajima's Neutrality Test for cattle populations from four locations in Taraba State are shown in Table 1, which uses nucleotide sequence data to study genetic variation patterns and neutral evolution deviations. The analyzed sequences across all locations showed similar results because they examined four to seven sequences and had 696 to 779 total sites. All studied populations showed high segregating sites (Ps) results which ranged from 0.887 to 0.991, which demonstrated substantial polymorphism between cattle populations in the entire research area. Watterson's theta (Θ), which estimates mutation rate through segregating sites, showed moderate values across locations, which indicates that all populations follow similar mutation patterns. Genetic variation estimation through this method remains strong for evaluating livestock genetic diversity across natural and human-driven breeding practices (Kardos et al., 2021). Nucleotide diversity (π) values in Donga reached 0.5759 while Gembu showed a higher value of 0.6781, which demonstrates moderate to high genetic diversity among both cattle populations. Populations with large effective population sizes show nucleotide diversity which results from multiple gene flow sources (Makina et al., 2020; Melka and Schenkel, 2021). Recent studies on African cattle breeds have documented comparable levels of nucleotide diversity which they attribute to admixture between indigenous and exotic breeds and to extensive pastoral mobility across agro-ecological zones (Smetko et al., 2021; Kim et al., 2020).

The most important finding shows that all tested locations show positive Tajima's D results which range from 1.99 at Donga to 4.04 at Gembu. Positive Tajima's D results show that alleles exist at intermediate frequencies which exceed the standard neutral model prediction, thus indicating that evolutionary processes operate outside of standard neutral evolution (Tajima, 1989; Cadzow et al., 2022). The observed pattern occurs because of balancing selection, population structure or admixture, or recent population bottlenecks (Charlesworth, 2020; Simonsen et al., 1995). The particularly high Tajima's D values recorded in Gembu and Wukari show that these populations exhibit stronger neutrality deviations because their cattle populations experience higher genetic background admixture and face environmental adaptation selective pressures. Recent genomic studies on West African cattle have identified significant population stratification driven by historical introgression events and adaptation to distinct climatic conditions, including heat tolerance and disease resistance (Tijjani et al., 2021;

Smetko et al., 2021; Mbole-Kariuki et al., 2020). The elevated Tajima's D in Gembu may also reflect adaptation to the cooler highland environment of the Mambilla Plateau, where selective pressures differ markedly from lowland areas (Bahbahani et al., 2021).

Donga showed the lowest Tajima's D value among all competitors, but it maintained a positive value which demonstrated that Donga experienced less intense evolutionary forces than other competitors. The presence of these factors indicates that the region has undergone more recent genetic mixing or experiences weaker selection pressures when compared to other areas (Nielsen et al., 2020). The results of the Tajima's Neutrality Test demonstrate that Taraba State cattle populations do not follow the pattern of strict neutral evolution. The population shows non-neutral evolution which is driven by two factors that include balancing selection and population subdivision according to different agro-ecological zones and cattle movements throughout the area. The research results support existing genomic studies on African cattle because they demonstrate that African cattle show intricate evolutionary patterns which include hybridization with local populations and changes in population size (Upadhyay et al., 2021; Kim et al., 2023; Porto-Neto et al., 2022). The study expects to achieve better genomic selection through the use of genome-wide SNP data because it will help researchers find specific genomic areas affected by selection and allow them to measure how demographic history and adaptive mechanisms operated in the past.

TABLE 1
RESULTS FROM TAJIMA'S NEUTRALITY TEST OF CATTLE BASED ON LOCATION IN TARABA STATE

Location	m	n	S	Ps	Θ	π	D
Iware	4	786	758	0.964	0.526	0.662	2.725
Wukari	7	785	778	0.991	0.405	0.635	3.334
Donga	4	784	696	0.888	0.484	0.576	1.989
Gembu	7	790	779	0.986	0.402	0.678	4.036

Abbreviations: m = number of sequences, n = total number of sites, S = Number of segregating sites, ps = S/n, Θ = ps/a1, π = nucleotide diversity, and D is the Tajima test statistic*

Table 2 displays the results of Tajima's Neutrality Test for the four tested cattle breeds which include Bokoloji, Muturu, Red Bororo, and White Fulani. The number of sequences analyzed (m) varied across breeds, ranging from 3 in Bokoloji and Muturu to 16 in White Fulani, reflecting differences in sampling intensity among breeds. The results show that all breeds demonstrate strong evidence of non-neutral evolution. This finding holds true despite limited sample sizes in Bokoloji and Muturu which decrease statistical power for testing results. The positive Tajima's D values which showed consistent results across all breeds demonstrate that non-neutral evolutionary patterns have produced strong evolutionary evidence.

The segregating site proportion showed moderate results in Bokoloji at 0.715 and in Muturu at 0.655. Red Bororo showed high polymorphism levels which reached 0.984 and White Fulani reached 0.997. Watterson's theta (Θ) showed moderate values across different breeds because it estimates population mutation rate from segregating sites, which showed higher values in Bokoloji and Muturu than in Red Bororo and White Fulani. The difference in Θ results from how allele frequencies change across different polymorphic levels according to Tajima (1989) and Kardos et al. (2021). The high number of segregating sites found in Red Bororo and White Fulani aligns with genomic research which shows that West African cattle breeds experienced extensive zebu and taurine genetic mixing (Kim et al., 2023; Weldenegker et al., 2024).

The study found genetic diversity within all breeds to range from 0.536 in Muturu to 0.699 in Red Bororo. The high π values in Red Bororo and White Fulani suggest that their effective population sizes remain large while their gene flow patterns resemble those found in widespread indigenous cattle breeds which receive extensive management (Melka & Schenkel, 2021; Upadhyay et al., 2021). Recent whole-genome sequencing studies have confirmed that Fulani zebu breeds including White Fulani and Red Bororo exhibit elevated genomic diversity attributable to historical introgression events and continued admixture with multiple cattle populations across the Sahel region (Tijjani et al., 2021; Bahbahani et al., 2021).

The nucleotide diversity of Muturu shows lower values which match the breed's status as an endangered trypanotolerant shorthorn taurine breed that exists only in limited areas and has a small breeding population base according to research by Tijjani and his team (2021) and Barbato and his team (2022). The conservation genomic research findings have detected

historic population bottlenecks together with genetic drift patterns in Muturu populations which require specific breeding initiatives to safeguard their genetic diversity according to Smetko et al. (2021) and Gobena et al. (2024). The breeds all showed positive Tajima's D values which proved their evolutionary patterns had changed from neutral genetic evolution. The D values of Bokoloji and Muturu showed moderately positive D values which indicated the presence of weak balancing selection together with mild population structure. The moderate deviations show two possible explanations which include either localized environmental adaptation or recent demographic changes which include founder effects and bottlenecks according to Charlesworth (2020) and Nielsen (2021).

The study found that Red Bororo and White Fulani populations displayed highly positive Tajima's D values which demonstrated their intermediate allele frequencies. The observed patterns result from balancing selection and population subdivision and genetically distinct population mixing instead of recent population growth (Tajima, 1989; Cadzow et al., 2022; Simonsen et al., 1995). The genomic studies conducted recently showed that Red Bororo and White Fulani West African zebu cattle possess adaptive introgression from both African taurine and indicine sources, which scientists found through selection analysis of genes linked to thermotolerance, disease resistance, and milk production (Porto-Neto et al., 2022; Bahbahani et al., 2021; Ginja et al., 2023). The genetic changes in Red Bororo and White Fulani show strong deviation from neutrality because of their ability to breed across various geographical areas, their genetic mixing patterns, and their various environmental and management circumstances. The two factors create conditions which enable organisms to maintain their genetic variation together with their special adaptive traits that support their capacity to thrive in diverse environments (Kim et al., 2023; Weldenegker et al., 2024). The research on African zebu cattle through genome-wide association studies (GWAS) discovered loci under balancing selection which govern innate immunity, heat shock protein control, and parasite resistance—the vital abilities needed for survival in tropical climates (Smetko et al., 2021; Zhou et al., 2023).

The high Tajima's D values in these breeds test present genetic patterns which continue to evolve through multiple ancestral connections. The population genomic studies revealed that modern Fulani cattle populations maintain genetic contact with their neighboring breeds, resulting in ancestral patterns that produce intermediate-frequency alleles throughout their genomic structure (Kim et al., 2023; Upadhyay et al., 2021; Barbato et al., 2022). This should explain why intermediate allele frequencies decrease through genetic drift and inbreeding in Muturu dog breeds which face strong selection for adaptive traits (Gobena et al., 2024). The study results show that non-neutral evolutionary processes determine genetic differences between the investigated cattle breeds, with widespread breeds showing stronger effects than local and genetically distinct breeding groups. The research results demonstrate that African indigenous cattle conservation and breeding programs need to include demographic history and admixture patterns and selection pressure as essential elements for their success (Weldenegker et al., 2024; Zhou et al., 2023). Future research using high-density SNP arrays together with whole-genome sequencing will enable researchers to identify selection patterns while uncovering how organisms adapt through genetic changes that violate natural selection rules (Ginja et al., 2023; Nielsen, 2021).

TABLE 2
RESULTS FROM TAJIMA'S NEUTRALITY TEST OF CATTLE BASED ON BREEDS

Breeds	m	n	S	Ps	Θ	π	D
Bokoloji	3	786	562	0.715	0.477	0.577	1.246
Muturu	3	782	512	0.655	0.436	0.536	1
Red Bororo	6	790	777	0.984	0.431	0.699	4.055
White Fulani	16	786	784	0.997	0.321	0.654	4.486

Abbreviations: m = number of sequences, n = total number of sites, S = Number of segregating sites, ps = S/n, Θ = ps/a1, π = nucleotide diversity, and D is the Tajima test statistic*

3.3 Tajima's Clock Test Results:

Table 3 displays Tajima's Clock Test results for four Taraba State cattle populations which were tested in Wukari, Iware, Donga, and Gembu. The test examines sequence divergence among three representative sequences from each population, providing insight into molecular clock consistency and evolutionary rate variation among sites (Tajima, 1993). This method enables researchers to identify molecular clock violations which occur when different selection pressures, population structure, and demographic changes take place (Kumar & Hedges, 2021; Lartillot and Poujol, 2024).

The quantity of matching sites which appeared in all three genetic sequences showed significant differences between various testing sites. Iware preserved 202 sites which represent its most conserved elements because its sequence patterns show lower divergence from other genetic materials. Donga and Gembu displayed intermediate numbers of identical sites (91 and 116, respectively). Geographically nearby groups show different genetic patterns which result from distinct population histories and different environmental pressures according to Kardos et al. (2021) and Charlesworth (2020). The highest number of shared genetic differences between three sequences occurred at 244 sites in Wukari and 229 sites in Donga, which demonstrated extensive genetic variation. Gembu had 193 divergent sites while Iware had only 6, which supports its high number of identical sites. The pattern shows that Wukari and Donga cattle populations experience more genetic changes than Iware cattle because their populations exist in more complex structures. Recent studies of African cattle genomes show that higher genetic differences between populations develop when distinct genetic groups interbreed, especially between zebu and taurine ancestors according to Kim et al. (2023) and Weldenegker et al. (2024). The high genetic differences found in Wukari and Donga indicate ongoing genetic exchange between different cattle populations which have different evolutionary backgrounds, a phenomenon that researchers have documented throughout West African pastoral systems according to Gobena et al. (2024) and Upadhyay et al. (2021).

The study of unique sequence differences enables researchers to examine how different genetic variants exist within the same population. The three sequences from Wukari showed different levels of unique genetic differences because sequence A had 137 differences, sequence B had 106 differences, and sequence C had 143 differences, which showed that private genetic mutations appeared evenly among the studied people. The Iware sequences showed lower unique differences because they maintained higher sequence conservation, which results in their lower unique differences (4–536 range). The Iware sequence contains one sequence with an extremely high value of 536, which probably represents either a data error or a highly distinct genetic variant that entered the population through breeding with another genetically different group, which needs to be confirmed through research that involves more samples (Cadzow et al., 2022). Gembu showed significant genetic diversity through its sequence C results, which produced 286 unique differences. The unique environmental conditions of the Mambilla Plateau highland, where Gembu exists, create cooler temperatures and higher altitudes and different disease threats, which enable local populations to develop distinct genetic traits (Bahbahani et al., 2021; Porto-Neto et al., 2022). East African highland cattle genetic studies found genes that control hypoxia adaptation, thermoregulation, and immune function, which Gembu cattle populations use for their adaptive processes (Smetko et al., 2021; Zhou et al., 2023).

The discovered differences in sequence divergence patterns show significant effects on how scientists use molecular clocks in their research. The divergence time estimates and phylogenetic reconstructions get impacted by clock-like evolution violations which cause evolutionary rate changes across different lineages and throughout various genomic areas (Kumar & Hedges, 2021; Lartillot & Poujol, 2024). The dataset requires advanced molecular clock models because Taraba State populations show distinct patterns of divergent sites and unique genetic differences. Future phylogenomic analyses need to use relaxed clock models which handle rate heterogeneity according to Álvarez-Carretero et al. (2022) and Tamuri et al. (2021). The research results show that Wukari and Donga cattle populations show more genetic diversity and sequence divergence, which Iware population shows through its higher genetic similarity. The three geographic locations show different patterns of genetic variation which Tajima (1993) and Nielsen (2021) identify as results from historical population movements, gene exchanges, environmental adaptation, and different evolutionary forces that operate in each location. Recent research on population genomics has shown that West African cattle populations underwent multiple demographic changes which included several rounds of genetic mixing together with population growth and adaptation to various agro-ecological regions (Kim et al., 2023; Tijjani et al., 2021; Barbato et al., 2022).

The variation that Tajima's Clock Test shows proves scientists need to study population structures for their practical work on estimating evolution rates and using molecular clock methods to study cattle genetics research. Future investigations should incorporate genome-wide SNP data or whole-genome sequences to comprehensively characterize population structure, admixture patterns, and selection signatures across these populations (Ginja et al., 2023; Weldenegker et al., 2024). The combination of environmental data with phenotypic information would allow researchers to find adaptive alleles while they study how environmental factors cause genetic differences among Taraba State cattle populations (Gobena et al., 2024; Melka & Schenkel, 2021). Evidence-based conservation and breeding strategies need integrative approaches to maintain adaptive genetic variation in indigenous African cattle while they work to enhance productivity (Zhou et al., 2023; Smetko et al., 2021).

TABLE 3
RESULTS FROM TAJIMA'S CLOCK TEST BASED ON LOCATION

Parameters	Wukari	Iware	Donga	Gembu
Identical sites in all three sequences	59	202	91	116
Divergent sites in all three sequences	244	6	229	193
Unique differences in sequence A	137	536	138	104
Unique differences in sequence B	106	5	151	78
Unique differences in sequence C	143	4	170	286

IV. CONCLUSION

The research presents a comprehensive assessment of genetic diversity and evolutionary relationships among Taraba State Nigerian cattle populations. The presence of positive Tajima's D values at all testing sites and among all tested breeds demonstrates that the species exhibit genetic patterns which differ from neutral evolution because of balancing selection, population structure, and historical demographic changes. Gembu and Wukari populations showed the highest nucleotide diversity and strongest signals of non-neutrality, reflecting substantial within-population variation. The breed-level study found that White Fulani and Red Bororo cattle showed higher genetic diversity and more pronounced neutrality violations than Bokoloji and Muturu because different factors affected their population size, geographic distribution, and environmental conditions. The Tajima's Relative Rate (Clock) Test showed different evolutionary rates across the study, as Iware maintained its basic sequences while showing irregular substitutions among its specific lineages, which broke the basic molecular clock rules. The phylogenetic study showed that breeds and geographical regions formed distinct groups, which scientists used to trace shared ancestry and population mixing. The research demonstrates how Nigerian cattle developed through complex evolutionary changes, which researchers used to create fundamental data needed for conservation work, breeding programs, and genetic development methods.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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