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# Short tandem repeat (STR) based genetic diversity and relationship of indigenous Niger cattle

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**Abstract.** The diversity of cattle in Niger is predominantly represented by three indigenous breeds: Zebu Arabe, Zebu Bororo and Kuri. This study aimed at characterizing the genetic diversity and relationship of Niger cattle breeds using short tandem repeat (STR) marker variations. A total of 105 cattle from all three breeds were genotyped at 27 STR loci. High levels of allelic and gene diversity were observed with an overall mean of 8.7 and 0.724 respectively. The mean inbreeding estimate within breeds was found to be moderate with 0.024, 0.043 and 0.044 in Zebu Arabe, Zebu Bororo and Kuri cattle respectively. The global $F$ statistics showed low genetic differentiation among Niger cattle with about 2.6 % of total variation being attributed to between-breed differences. Neighbor-joining tree derived from pairwise allele sharing distance revealed Zebu Arabe and Kuri clustering together while Zebu Bororo appeared to be relatively distinct from the other two breeds. High levels of admixture were evident from the distribution of pairwise inter-individual allele sharing distances that showed individuals across populations being more related than individuals within populations. Individuals were assigned to their respective source populations based on STR genotypes, and the percent correct assignment of Zebu Bororo (87.5 to 93.8 %) was consistently higher than Zebu Arabe (59.3 to 70.4 %) and Kuri (80.0 to 83.3 %) cattle. The qualitative and quantitative tests for mutation drift equilibrium revealed absence of genetic bottleneck events in Niger cattle in the recent past. High genetic diversity and poor genetic structure among indigenous cattle breeds of Niger might be due to historic zebu–taurine admixture and ongoing breeding practices in the region. The results of the present study are expected to help in formulating effective strategies for conservation and genetic improvement of indigenous Niger cattle breeds.

## 1 Introduction

Niger is a landlocked sub-Saharan country in West Africa that primarily depends on agriculture and livestock. The livestock sector contributes about 40 % of the country's agricultural gross domestic product (GDP) and 14 % of the national GDP (Apaa-Okello et al., 2015). The rural livelihood is essentially dependent on livestock with four out of every five households rearing animals. Each of these households owns about 2.8 Tropical Livestock Units (TLU) that are predominantly made up of cattle (55 %) and small ruminants (33 %) (World Bank, 2012). Cattle in Niger not only play a vital role in rural subsistence but also possess significant cultural importance, particularly during the performance of religious rites and celebrations. The cattle population in Niger num-

bers around 11.4 million (FAOSTAT, 2014) and forms part of the primary non-mineral exports from the country. Most of these cattle belong to indigenous breeds/populations that are well adapted to survive and reproduce under harsh Sahelian conditions, often affected by varied rainfall and drought (Okomo-Adhiambo, 2002). Documentation of these native breeds has been mostly limited to physical or zootechnic characteristics with little or no information available on their genetic and production performance characteristics.

Indigenous cattle of Niger include zebu breeds like Azawak, Bororo, Djelli/Arabe and Goudali as well as a taurine population called Kuri. Kuri (also known as the Baharie, Bare, Borrie, Boudouma, Dongolé, Koubouri, Buduma) is a large-bodied humpless longhorn cattle whose exact historical origin is unknown (Blench, 1993; Meghen et al., 2000). These cattle are distributed in the region of Lake Chad and along its eastern shores. Kuri cattle has distinctive, inflated, spongy horns with a mean height of 1.5 m at withers and an average estimated body weight of about 550 kg. Kuri are one of the largest breeds of African cattle and are noted for their extremely variable coat color pattern, meat and milk production potentials and ability to thrive under semi-aquatic conditions. The breed is well adapted to the semi-aquatic environment of Lake Chad such that it is unable to survive elsewhere. Kuri cattle is currently classified under the category threatened by extinction.

Bororo, also called Red Fulani, Red Bororo, Wodabe, Fellata, Foulata, Abori, Bodadi, Brahaza and Djafoun, are one of the largest zebu breeds in West Africa. They are distinguished by deep burgundy colored coat, pendulous ears and long, thick lyre-shaped horns that can extend up to 120–140 cm. These are temperamental, intractable cattle suitable to arid and semi-arid regions, but are poor milkers. They are perfectly adapted to long marches and are therefore well suitable for transhumant pastoral systems. At birth, both male and female calves weigh between 15 and 20 kg and, under natural conditions, the adult weight ranges from 400 to 500 kg for males and 350 to 450 kg for females. Weights vary widely according to the seasonal availability and quality of fodder in the region. In Niger, Zebu Bororo is mainly located in the southern part of the country, particularly in Dosso, Tahoua, Maradi and Diffa regions. Around Diffa, Bororo cattle are distributed together with the taurine type Kuri cattle. Zebu Arabe cattle (known as Arab Shuwa, Arab Choa or Wadera) are also located in the area surrounding Lake Chad overlapping the habitat of Kuri cattle. They are medium-sized and lightly built animals. They usually possess dark red, black, pied or brown coat color with or without small white patches on the underline. Zebu Arabe are short-horned cattle and have a small erect hump. The ears are long but not pendulous with short, moderately thick, round or flat horns. Their usefulness under stressful environment has been well recognized; the animals are considered to be good dairy animals and used by women for riding and as pack animals. Information on genetic diversity and relationship among indigenous cattle of Niger is very limited. Genetic diversity is an essential component in the fitness of a population, for it to survive and adapt to the changing environmental conditions. Molecular characterization of indigenous breeds is the primary step in formulating and optimizing strategies for conservation and genetic improvement, to increase productivity as well as meeting future market demands. The present study was undertaken to evaluate genetic diversity of three Niger cattle breeds/populations (Kuri, Zebu Bororo and Zebu Arabe) and to assess their genetic relationship using multilocus short tandem repeat data.

## 2 Materials and methods

### 2.1 Sampling and STR genotyping

A total of 105 individuals belonging to three Niger cattle breeds, two Zebus (Arabe and Bororo) and one taurine (Kuri) were sampled. The sampling site included shores and islands of Lake Chad situated at the east of the arid Sahel agro-ecological region between 12°20′–14°20′ N latitude and 13–15°30′ E longitude and bordered by Cameroon, Chad and Nigeria. The numbers of animals sampled for each of the three cattle breeds were as follows: Zebu Arabe (27), Zebu Bororo (48) and Kuri (30). The farmers were interviewed in detail to ensure the unrelatedness of sampled cattle. Blood samples were collected after jugular venipuncture in EDTA coated Vacutainer tubes, and DNA was extracted from whole blood using MasterPure DNA Purification Kit (Biozym, Illumina Inc, USA). DNA samples were then stored at 4 °C until PCR amplification and genotyping. 27 FAO-recommended short tandem repeat (STR) markers (FAO, 2011) with forward primers conjugated to one of the three fluorescent dyes (FAM, HEX and ATTO550) were used for diversity analysis. Polymerase chain reaction was performed under the following conditions: initial denaturation for 5 min at 95 °C, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at respective temperature of each marker locus for 1 min, elongation at 72 °C for 1 min with a final extension at 72 °C for 10 min. The PCR products were then electrophoresed after multiplexing in an automated DNA analyzer ABI3100 (Applied Biosystems, USA) with ROX500 (Applied Biosystems, USA) as an internal lane control. All 27 STR loci were multiplexed in six sets for genotyping, as shown in Table 1. The allele size data for each sample were then extracted using GeneMapper v.4.1 software (Applied Biosystems, USA).

### 2.2 Statistical analysis of STR data

The presence of null alleles in the dataset was checked using MicroChecker version 2.2.3 (Oosterhout et al., 2004). Basic diversity indices like observed number of alleles, observed and expected heterozygosity, pairwise and global $F$ statistics were calculated using Microsatellite Analyzer (MSA) version 3.15 (Dieringer and Schlötterer, 2003). The effective

**Table 1.** Details of microsatellite loci and allelic diversity in Zebu Arabe, Zebu Bororo and Kuri breeds of Niger cattle.

| Locus | Multiplex panel | Anneal temp. | Dye | Allele size range | Zebu Arabe | | Zebu Bororo | | Kuri | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | $n_a$ | $n_e$ | $n_a$ | $n_e$ | $n_a$ | $n_e$ |
| CSRM60 | 1 | 60 °C | FAM | 87–109 | 10 | 3.83 | 10 | 2.65 | 7 | 3.64 |
| CSSM66 | 1 | 60 °C | FAM | 177–197 | 9 | 6.47 | 10 | 6.32 | 8 | 4.77 |
| HEL1 | 1 | 56 °C | HEX | 96–114 | 5 | 3.24 | 5 | 3.04 | 8 | 3.53 |
| INRA63 | 1 | 56 °C | HEX | 173–183 | 4 | 1.85 | 4 | 2.02 | 5 | 2.57 |
| BM1824 | 2 | 61 °C | ATTO550 | 183–197 | 4 | 3.54 | 4 | 3.79 | 4 | 2.48 |
| ETH152 | 2 | 60 °C | FAM | 189–199 | 6 | 2.31 | 6 | 2.39 | 4 | 2.24 |
| HAUT27 | 2 | 54 °C | HEX | 140–150 | 4 | 2.76 | 6 | 3.74 | 4 | 2.62 |
| INRA05 | 2 | 54 °C | FAM | 134–148 | 6 | 2.96 | 4 | 2.16 | 5 | 2.70 |
| BM1818 | 3 | 60 °C | HEX | 256–272 | 7 | 4.89 | 8 | 3.96 | 7 | 4.36 |
| ETH3 | 3 | 63 °C | FAM | 99–125 | 6 | 3.09 | 7 | 1.86 | 5 | 2.86 |
| HEL9 | 3 | 56 °C | ATTO550 | 155–175 | 11 | 7.11 | 10 | 6.49 | 9 | 6.44 |
| ILSTS006 | 3 | 54 °C | FAM | 284–300 | 7 | 3.50 | 8 | 3.38 | 7 | 2.60 |
| TGLA53 | 3 | 55 °C | HEX | 153–187 | 14 | 4.92 | 14 | 4.56 | 11 | 5.86 |
| HAUT24 | 4 | 53 °C | HEX | 103–125 | 7 | 5.81 | 8 | 4.12 | 6 | 4.56 |
| HEL5 | 4 | 54 °C | FAM | 148–164 | 5 | 3.35 | 7 | 4.75 | 7 | 3.97 |
| INRA032 | 4 | 56 °C | ATTO550 | 164–208 | 8 | 6.13 | 7 | 5.34 | 7 | 4.23 |
| SPS115 | 4 | 61 °C | FAM | 243–255 | 5 | 2.34 | 6 | 1.79 | 7 | 2.39 |
| ETH185 | 5 | 65 °C | ATTO550 | 224–252 | 9 | 4.88 | 11 | 2.97 | 8 | 3.73 |
| HEL13 | 5 | 54 °C | HEX | 176–194 | 6 | 3.85 | 5 | 3.13 | 6 | 3.99 |
| ILSTS05 | 5 | 56 °C | FAM | 178–190 | 5 | 3.37 | 5 | 3.76 | 5 | 3.61 |
| INRA035 | 5 | 60 °C | FAM | 99–123 | 5 | 2.06 | 8 | 3.35 | 7 | 2.89 |
| TGLA126 | 5 | 54 °C | HEX | 114–128 | 7 | 3.25 | 7 | 3.40 | 8 | 5.52 |
| BM2113 | 6 | 63 °C | FAM | 118–146 | 8 | 6.34 | 9 | 5.90 | 8 | 6.57 |
| ETH10 | 6 | 61 °C | FAM | 207–225 | 8 | 5.03 | 7 | 3.99 | 6 | 2.34 |
| ETH225 | 6 | 63 °C | ATTO550 | 140–162 | 7 | 2.66 | 8 | 3.38 | 7 | 2.85 |
| INRA023 | 6 | 58 °C | ATTO550 | 199–219 | 9 | 3.65 | 8 | 2.90 | 7 | 4.79 |
| TGLA122 | 6 | 58 °C | HEX | 134–174 | 10 | 3.99 | 8 | 2.56 | 9 | 4.11 |
| Mean | – | – | – | – | 7.11 | 3.97 | 7.41 | 3.62 | 6.74 | 3.79 |

number of alleles per locus was estimated using GenAlEx 6.503 (Peakall and Smouse, 2012). Deviations of heterozygosities from Hardy–Weinberg equilibrium (HWE) were estimated by (1) calculating the degree of within-population reduction in heterozygosity ($F_{IS}$) following Wright (1951) and permutational test for heterozygosity deficit with jackknifing over loci using FSTAT 2.9.3. Goudet, 2001) and with (2) exact tests of heterozygote excess for each marker and in each breed, as implemented in Genepop (Raymond and Rousset, 1995). The neutrality of the microsatellites used in this study was evaluated by comparing the markers against neutral expectations in a distribution of $F_{ST}$ vs. heterozygosities under an island model of migration using LOSITAN version 1 (Antao et al., 2008). Pairwise allele sharing distance between populations and inter-individual allele sharing distances were also calculated using MSA. Pairwise inter-individual allele sharing distance was utilized to construct the circular tree following neighbor-joining algorithm using PHYLIP version 3.5 (Felsenstein, 1993) and the tree was visualized using MEGA version 6.0 (Tamura et al., 2013). To ascertain the recent history of Niger cattle breeds, probabilities of assignment were computed based on likelihood (Paetkau et al., 1995) and Bayesian (Rannala and Mountain, 1997; Baudouin and Lebrun, 2001) methods as implemented in GeneClass 2 software (Piry et al., 2004). The scatter plot of log-likelihood estimates (Baudouin and Lebrun, 2001) for individuals of each of the three Niger cattle breeds was displayed using SPSS version 10.5. The Niger cattle populations were tested for mutation drift equilibrium following three statistical approaches (sign test, standardized differences test and Wilcoxon sign rank test) under different models of microsatellite evolution as implemented in the BOTTLENECK program (Piry et al., 1999).

## 3 Results and discussion

### 3.1 Genetic variation in Niger cattle

A total of 2835 genotypes from 105 animals across 27 microsatellite marker loci were utilized for the present study. The MicroChecker analysis of Niger cattle genotypes at all loci revealed no significant presence of null alleles. In total, 235 alleles were amplified across 27 STR loci in all three

**Table 2.** Observed heterozygosity ($H_o$), expected heterozygosity ($H_e$) and estimated heterozygosity deficit ($F_{IS}$) at different loci in Zebu Arabe, Zebu Bororo and Kuri breeds of Niger cattle.

| Locus | Zebu Arabe | | | Zebu Bororo | | | Kuri | | |
|---|---|---|---|---|---|---|---|---|---|
| | $H_o$ | $H_e$ | $F_{IS}$ | $H_o$ | $H_e$ | $F_{IS}$ | $H_o$ | $H_e$ | $F_{IS}$ |
| CSRM60 | 0.750 | 0.754 | 0.006* | 0.617 | 0.629 | 0.019 | 0.759 | 0.738 | −0.028 |
| CSSM66 | 0.792 | 0.863 | 0.085** | 0.844 | 0.851 | 0.008 | 0.700 | 0.804 | 0.131 |
| HEL1 | 0.731 | 0.705 | −0.037 | 0.565 | 0.679 | 0.169 | 0.700 | 0.729 | 0.04 |
| INRA63 | 0.458 | 0.469 | 0.023 | 0.543 | 0.509 | −0.068 | 0.667 | 0.621 | −0.074 |
| BM1824 | 0.808 | 0.732 | −0.106 | 0.667 | 0.744 | 0.104 | 0.633 | 0.607 | −0.045 |
| ETH152 | 0.538 | 0.578 | 0.069 | 0.563 | 0.587 | 0.042 | 0.633 | 0.563 | −0.127 |
| HAUT27 | 0.481 | 0.649 | 0.262 | 0.702 | 0.741 | 0.053 | 0.379 | 0.629 | 0.401** |
| INRA05 | 0.778 | 0.675 | −0.156 | 0.521 | 0.542 | 0.04 | 0.567 | 0.641 | 0.117 |
| BM1818 | 0.741 | 0.811 | 0.088 | 0.681 | 0.755 | 0.1 | 0.655 | 0.784 | 0.167 |
| ETH3 | 0.593 | 0.689 | 0.142 | 0.417 | 0.468 | 0.111 | 0.643 | 0.662 | 0.029 |
| HEL9 | 0.926 | 0.876 | −0.059 | 0.870 | 0.855 | −0.017 | 0.966 | 0.860 | −0.126 |
| ILSTS006 | 0.852 | 0.728 | −0.174 | 0.745 | 0.712 | −0.047 | 0.621 | 0.626 | 0.009 |
| TGLA53 | 0.769 | 0.812 | 0.054 | 0.771 | 0.789 | 0.023 | 0.793 | 0.844 | 0.061 |
| HAUT24 | 0.815 | 0.843 | 0.035 | 0.625 | 0.765 | 0.185 | 0.700 | 0.794 | 0.12 |
| HEL5 | 0.556 | 0.715 | 0.226** | 0.652 | 0.798 | 0.185* | 0.464 | 0.762 | 0.395** |
| INRA032 | 0.778 | 0.853 | 0.089 | 0.813 | 0.821 | 0.011 | 0.800 | 0.776 | −0.031 |
| SPS115 | 0.630 | 0.584 | −0.081 | 0.465 | 0.448 | −0.04 | 0.655 | 0.592 | −0.108 |
| ETH185 | 0.885 | 0.811 | −0.093 | 0.581 | 0.671 | 0.135 | 0.571 | 0.745 | 0.237* |
| HEL13 | 0.667 | 0.754 | 0.118 | 0.652 | 0.688 | 0.053 | 0.690 | 0.762 | 0.097 |
| ILSTS05 | 0.692 | 0.717 | 0.035 | 0.694 | 0.745 | 0.068 | 0.700 | 0.735 | 0.048 |
| INRA035 | 0.444 | 0.523 | 0.153* | 0.630 | 0.709 | 0.112 | 0.448 | 0.665 | 0.33* |
| TGLA126 | 0.704 | 0.705 | 0.002 | 0.689 | 0.714 | 0.036 | 0.967 | 0.833 | −0.164 |
| BM2113 | 0.852 | 0.858 | 0.007 | 0.896 | 0.839 | −0.068 | 0.900 | 0.862 | −0.045 |
| ETH10 | 0.852 | 0.816 | −0.045 | 0.809 | 0.757 | −0.068 | 0.567 | 0.583 | 0.029 |
| ETH225 | 0.556 | 0.636 | 0.128 | 0.708 | 0.711 | 0.004 | 0.733 | 0.660 | −0.112 |
| INRA023 | 0.815 | 0.739 | −0.104 | 0.682 | 0.663 | −0.029 | 0.900 | 0.805 | −0.121 |
| TGLA122 | 0.778 | 0.764 | −0.019 | 0.583 | 0.616 | 0.053 | 0.800 | 0.769 | −0.04 |
| Mean | 0.713 | 0.728 | 0.024** | 0.666 | 0.697 | 0.043** | 0.689 | 0.720 | 0.044** |

* $P < 0.05$; ** $P < 0.01$

cattle breeds of Niger indicating a high level of allelic diversity. The overall observed number of alleles ranged from 4 (BM1824) to 17 (TGLA53) with a mean of 8.7. Within breeds, the mean observed number of alleles per locus was 7.11, 7.41 and 6.74 in Zebu Arabe, Zebu Bororo and Kuri cattle respectively (Table 1). The overall mean observed and expected heterozygosity in Niger cattle was 0.685 and 0.724 respectively. The overall observed heterozygosity ranged from 0.524 (ETH3) to 0.912 (HEL9), while the overall expected heterozygosity varied between 0.533 (SPS115) and 0.869 (HEL9) across the investigated loci. The mean observed heterozygosity was 0.713, 0.666 and 0.689 in Zebu Arabe, Zebu Bororo and Kuri cattle respectively, while the mean expected heterozygosity was estimated to be 0.728, 0.697 and 0.720 respectively (Table 2). Among the three Niger cattle breeds, allelic diversity was highest in Zebu Bororo cattle, while the heterozygosity estimates were observed to be highest in Zebu Arabe cattle. Estimation of effective number of alleles revealed higher values in Zebu Arabe ($n_e = 3.97$) and Kuri ($n_e = 3.79$) breeds as compared to Zebu Bororo ($n_e = 3.62$) cattle. Effective number of alleles is the number of alleles with equal frequencies that would be necessary to achieve the same level of expected heterozygosity ($H_e$) in the study population. The values observed in Niger cattle are understandable as the sample size varied across the breeds and low-frequency alleles normally contribute little to the effective number of alleles. Estimates of allelic diversity and heterozygosity in Niger cattle breeds were comparable to those of Cameroonian (Ngono Ema et al., 2014), Senegal (Ndiaye et al., 2015), Sahelian (Alvarez et al., 2014), Sudanese (Hussein et al., 2015), Ethiopian (Dadi et al., 2008) and West and Central African Zebu (Ibeagha-Awamu et al., 2004) cattle. However, these estimates were higher than reported for Mozambican (Bessa et al., 2009), West and Central African taurine cattle (Ibeagha-Awamu et al., 2004). In general, the indigenous cattle breeds of Niger showed high levels of genetic diversity, possibly due to admixture of zebu and taurine

cattle that had formed the foundation stock of these populations in the past (Dadi et al., 2008).

### 3.2 Test for Hardy–Weinberg equilibrium

The overall mean inbreeding estimate ($F_{IS}$) was 0.051 and it varied between −0.075 (INRA023) and 0.257 (HEL5). The mean inbreeding estimate within breeds was 0.024, 0.043 and 0.044 in Zebu Arabe, Zebu Bororo and Kuri cattle respectively (Table 2). All three cattle breeds of Niger showed significantly positive mean $F_{IS}$ indicating moderate heterozygosity deficit. Locus-wise comparison of observed and expected heterozygosity within breeds showed heterozygosity deficit in 17, 20 and 15 loci in Zebu Arabe, Zebu Bororo and Kuri cattle respectively. However, heterozygosity excess was observed in 10, 7 and 12 loci in the three breeds respectively. The test for Hardy–Weinberg equilibrium (HWE) revealed significant deviations ($P > 0.05$) for heterozygosity deficiency at 4, 1 and 4 loci in Zebu Arabe, Zebu Bororo and Kuri cattle respectively (Table 2). Of these, locus HEL5 deviated significantly in all three breeds of cattle. A total of 21 out of the 27 investigated loci did not deviate from HWE ($P > 0.05$) in all three cattle breeds studied. HWE test for heterozygosity excess revealed significant deviations in 1 and 2 loci in Zebu Arabe and Kuri cattle respectively. No significant deviations for heterozygosity excess was observed in Zebu Bororo cattle. To summarize, out of 81 breed × locus combinations tested in Niger cattle, 11.1 % deviated significantly from HWE due to heterozygosity deficit, while 3.7 % deviated significantly due to heterozygosity excess. Departure from HWE due to heterozygosity deficit may result from one or more of the following reasons: (i) presence of null alleles, (ii) intense artificial selection and/or use of few breeding bulls in the region, (iii) selective forces operating at certain loci, (iv) non-random sampling and age structure of samples used, (v) assortative mating, (vi) sex linkage and (vii) Wahlund effect, i.e., presence of fewer heterozygotes in a population than predicted on account of population subdivision (Waples, 2015). The role of null alleles for the observed heterozygosity deficit can be discounted based on the results of MicroChecker analysis. Breeding of native Niger cattle is mostly done through natural service with very low coverage of cows under artificial insemination every year. Selection of animals for improved productivity is rarely practiced and hence reduction in heterozygosity due to intense artificial selection and use of few breeding sires do not arise. Further, the test for selective neutrality following $F_{ST}$ outlier approach as implemented in LOSITAN revealed no significant deviations in all 27 marker loci investigated. Population subdivision could be one of the possible causes for the observed heterozygosity deficit considering the fact that samples were collected from different geographical locations of the native breed tract. However, it also needs to be mentioned that much higher deviations from HWE have been reported in Brazilian (19.5 % breed × locus combinations; Egito et al., 2007) and Indian zebu cattle (17.3 % breed × locus combinations; Sharma et al., 2015).

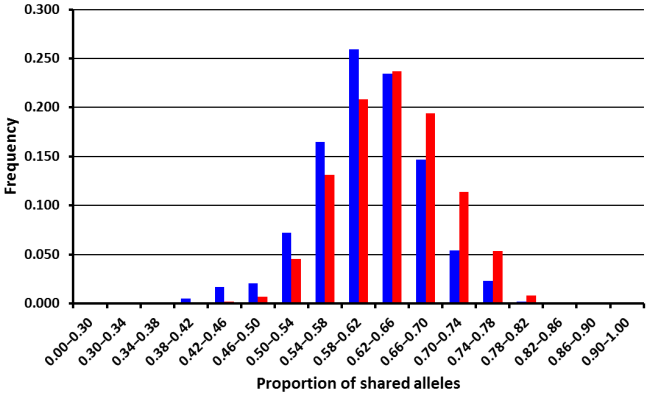


**Figure 1.** Distribution of allele sharing distance (IBS) between pairs of individuals. Distance was plotted separately where pairs were drawn from within the same breed (blue bars) and from across the breeds (red bars).

**Table 3.** Pairwise allele sharing distance (lower triangle) and pairwise $F_{ST}$ (upper triangle) among three Niger cattle breeds.

| | Zebu Arabe | Bororo | Kuri |
|---|---|---|---|
| Zebu Arabe | – | 0.021** | 0.016 |
| Zebu Bororo | 0.234 | – | 0.035** |
| Kuri | 0.223 | 0.259 | – |

** $P < 0.01$

### 3.3 Genetic differentiation among Niger cattle breeds

The mean global $F_{ST}$ ranged from −0.003 (BM1818) to 0.166 (ETH3) among different STR loci with an estimated mean value of 0.026 ($P < 0.01$), indicating 2.6 % of the total variation being attributed to between-breed differences. Although the overall mean global $F_{ST}$ was significant, 10 out of 27 investigated loci did not have significant $F_{ST}$ ($P > 0.05$) indicating low genetic differentiation among the Niger cattle breeds. The pairwise $F_{ST}$ between breeds ranged from 0.016 (Zebu Arabe–Kuri) to 0.035 (Zebu Bororo–Kuri). Similarly, the pairwise allele sharing distance varied from 0.223 (Zebu Arabe–Kuri) to 0.259 (Zebu Bororo–Kuri) (Table 3). The overall $F_{ST}$ observed in Niger cattle was much lower than that reported for Sudanese ($F_{ST} = 0.084$; Hussein et al., 2015), Cameroonian ($F_{ST} = 0.061$; Ngono Ema et al., 2014) and other West African cattle ($F_{ST} = 0.06$; Freeman et al., 2004), while the estimate was relatively higher than that of Ethiopian cattle ($F_{ST} = 0.013$; Dadi et al., 2008). Further, to evaluate within- and between-population diversities, pairwise allele sharing distances were calculated for all possible pairs of individuals within and across populations. The average inter-individual distance within breeds was estimated to

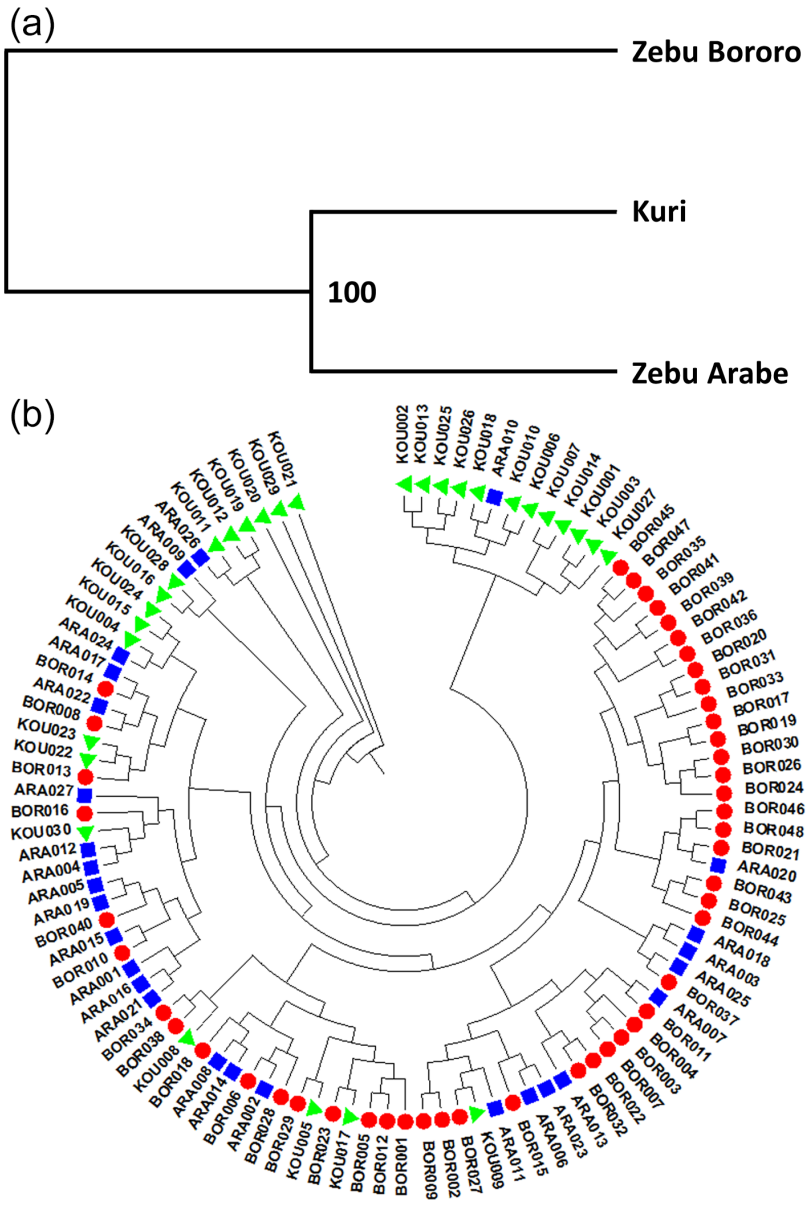


**Figure 2.** Neighbor-joining tree based on pairwise **(a)** population and **(b)** inter-individual allele sharing distances among Zebu Arabe (ARA), Zebu Bororo (BOR) and Kuri (KOU) breeds of Niger cattle (value at the node in **a** indicates percent bootstrap value out of 1000 resampled subsets of data).

be 0.624, 0.601 and 0.619 in Zebu Arabe, Zebu Bororo and Kuri respectively. The global average distance between individuals drawn from the same population was estimated to be 0.609, while the average distance between individuals drawn from different populations was estimated to be 0.637.

The distribution of inter-individual allele sharing distances from within and across populations appeared to be normal (Fig. 1). Further, a large overlap was observed in the distribution of inter-individual distances estimated from within and between breeds indicating that individuals across populations were more related than individuals within populations. Neighbor-joining tree derived from pairwise allele sharing distance revealed Zebu Arabe and Kuri clustering together, while Zebu Bororo appeared to be relatively distinct from them (Fig. 2a). However, neighbor-joining tree derived from pairwise inter-individual allele sharing distances revealed admixture of individuals from all three breeds, although a subset of individuals from Kuri and Zebu Bororo clustered distinctly (Fig. 2b). This is understandable considering the level of zebu–taurine crossbreeding that has been occurring in the region. Particularly, livestock owners in the region of Lake Chad prefer Kuri × Zebu crossbred cattle primarily because

**Table 4.** Genotype assignment of Zebu Arabe, Zebu Bororo and Kuri breeds of Niger cattle.

| Breed | *N* | Paetkau et al. (1995) | | Rannala and Mountain (1997) | | Baudouin and Lebrun (2001) | |
|---|---|---|---|---|---|---|---|
| | | No. correctly assigned | % correctly assigned | No. correctly assigned | % correctly assigned | No. correctly assigned | % correctly assigned |
| Zebu Arabe | 27 | 16 | 59.3 | 19 | 70.4 | 18 | 66.7 |
| Zebu Bororo | 48 | 43 | 89.6 | 42 | 87.5 | 45 | 93.8 |
| Kuri | 30 | 24 | 80.0 | 24 | 80.0 | 25 | 83.3 |
| Total | 105 | 83 | 79.0 | 85 | 81.0 | 88 | 83.8 |

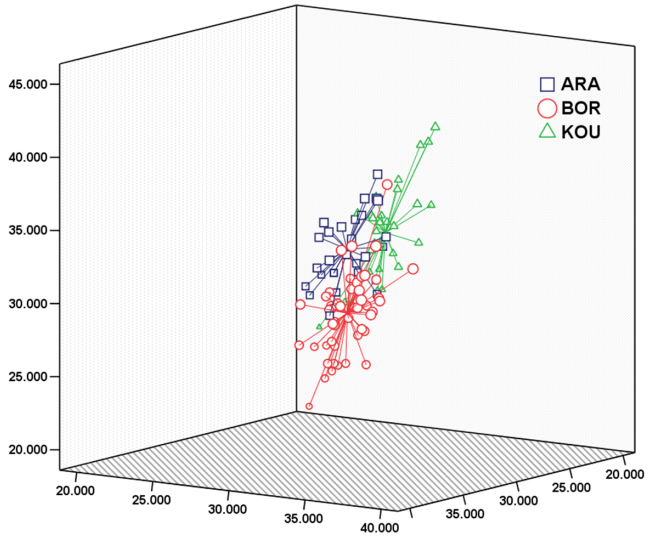


**Figure 3.** Scatter plot of log-likelihood estimates (Baudouin and Lebrun, 2001) for different individuals of Zebu Arabe (ARA), Zebu Bororo (BOR) and Kuri (KOU) breeds of Niger cattle.

of their large size, relatively higher milk yield, greater fertility and enhanced draught ability. Combined with the reducing habitat due to retreating waters of Lake Chad, Kuri cattle population has declined sharply despite their unique characteristics of being adapted to a semi-aquatic production environment. In case of Zebu Bororo, animals are distributed in a much wider geographical range as compared to Kuri and Zebu Arabe cattle. Clustering of a subset of Zebu Bororo cattle indicates population subdivision; however a larger number of animals needs to be sampled from different regions to confirm the existence of such a cryptic genetic structure.

### 3.4 Individual assignment in Niger cattle

Assignment methods using prior information to ascertain population membership of individuals or groups of individuals have been utilized to assess genetic structure in population genetic studies (Manel et al., 2005). Assignment tests are based on multi-locus genetic data and use both individual genotypes and population-level allele frequencies. In the present study, allele frequencies were utilized to perform genotype assignment based on likelihood and Bayesian methods. The former method calculated the likelihood of drawing a single multi-locus genotype from several potential sources based on the observed allele frequencies at each locus in each source. Genotype assignment following Bayesian procedure involved computation of likelihood of a genotype in a given population under the assumption of equal prior probability density to the allelic frequencies of each locus in each population. This method showed better assignment performance than frequency-based methods in simulated as well as real populations (Cornuet et al., 1999; Arranz et al., 2001; Legaz et al., 2008). Individuals were assigned to their respective source populations, and the percent correct assignment of Zebu Bororo (87.5 to 93.8 %) was consistently higher than Zebu Arabe (59.3 to 70.4 %) and Kuri (80.0 to 83.3 %) cattle across the three evaluated methods (Table 4). Further, plotting of log-likelihood estimates of individual animals in a scattergram revealed overlapping clusters with intermixing of individuals from each of the three cattle breeds (Fig. 3). High levels of admixture in Niger cattle were strongly evident and consistent with the reports on other African cattle (Dadi et al., 2008; Ndiaye et al., 2015). A general point of view is that the *B. indicus* first entered the African continent via the Suez route from Arabia (Epstein and Mason, 1984) and maritime routes to the Horn of Africa (Hanotte et al., 2002). The introduced zebu cattle intermingled and crossbred with the original African taurine to produce the various types of cattle found in East Africa today (Payne and Wilson, 1999). The current breeds/populations of West African zebu (including Zebu Arabe and Zebu Bororo breeds) might have resulted due to migrations of pastoralists from east to west (Clutton-Brock, 1989; Hanotte et al., 2002). The historic gene flow combined with ongoing breeding practices in the region apparently resulted in high levels of admixture among Niger cattle.

### 3.5 Test for mutation drift equilibrium

Populations that have experienced a recent reduction in their effective size exhibit a correlative reduction of allele numbers and gene diversity. For highly variable loci like microsatellites, the rare alleles (alleles with extremely low frequency)

**Table 5.** Tests for mutation drift equilibrium in Zebu Arabe, Zebu Bororo and Kuri breeds of Niger cattle.

| Test | | Arabe | | | Bororo | | | Kuri | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | IAM | TPM | SMM | IAM | TPM | SMM | IAM | TPM | SMM |
| Sign test | Expected no. of loci with $H_e$ excess | 16.14 | 16.20 | 16.14 | 16.06 | 16.11 | 15.97 | 16.09 | 16.14 | 16.10 |
| | Observed no. of loci with $H_e$ excess | 15 | 13 | 9 | 16 | 12 | 7 | 18 | 12 | 8 |
| | $P$ value | 0.396 | 0.145 | 0.005 | 0.563 | 0.079 | 0.001 | 0.294 | 0.077 | 0.002 |
| Standardized differences test | $T_2$ value | −0.29 | −0.32 | −7.62 | 0.19 | −3.89 | −10.39 | 1.00 | −1.67 | −5.94 |
| | $P$ value | 0.386 | 0.001 | 0.000 | 0.423 | 0.000 | 0.000 | 0.189 | 0.048 | 0.000 |
| Wilcoxon sign rank test | $P$ value (one tail for $H_e$ excess) | 0.420 | 0.944 | 0.998 | 0.297 | 0.922 | 0.999 | 0.101 | 0.860 | 0.999 |

are expected to be lost quickly, whereas gene diversity will be lost more slowly. This causes a transient excess of gene diversity after the bottleneck, in comparison to the value expected on the basis of the number of alleles in the population. This temporary genetic signature observed in experimental and wild populations that experienced recent bottleneck events provides a means of detecting recent reductions in effective population size (Shama et al., 2011). Therefore, the reduction in population size (e.g., Kuri) and excess of observed heterozygosity (10, 7 and 12 loci in Zebu Arabe, Zebu Bororo and Kuri cattle respectively) at most of the investigated loci formed the basis to evaluate Niger cattle breeds for mutation drift equilibrium (Kataria et al., 2010; Ganapathi et al., 2012). Niger cattle breeds were subjected to three different statistical tests for mutation drift equilibrium, viz. sign test, Wilcoxon sign rank test and standardized differences test. For each of these tests, three different models of microsatellite evolution – infinite allele model (IAM), stepwise mutation model (SMM) and two-phase model (TPM) – were assumed. All three statistical tests revealed no significant deviation from mutation drift equilibrium, indicating absence of genetic bottleneck in Niger cattle breeds (Table 5). Standardized differences test showed negative $T_2$ values for all three breeds under different mutation models (except for Zebu Bororo and Kuri under IAM), clearly indicating the absence of significant heterozygosity excess. In addition, the qualitative method based on mode-shift distortion also showed a normal L-shaped curve in all three Niger cattle breeds (Fig. 4). The frequency distribution of alleles showed abundance of low-frequency alleles (0.001 to 0.1) and no significant loss of rare alleles was observed. Thus, both quantitative and qualitative analysis indicated that the Niger cattle did not experience any genetic bottleneck in the recent past.

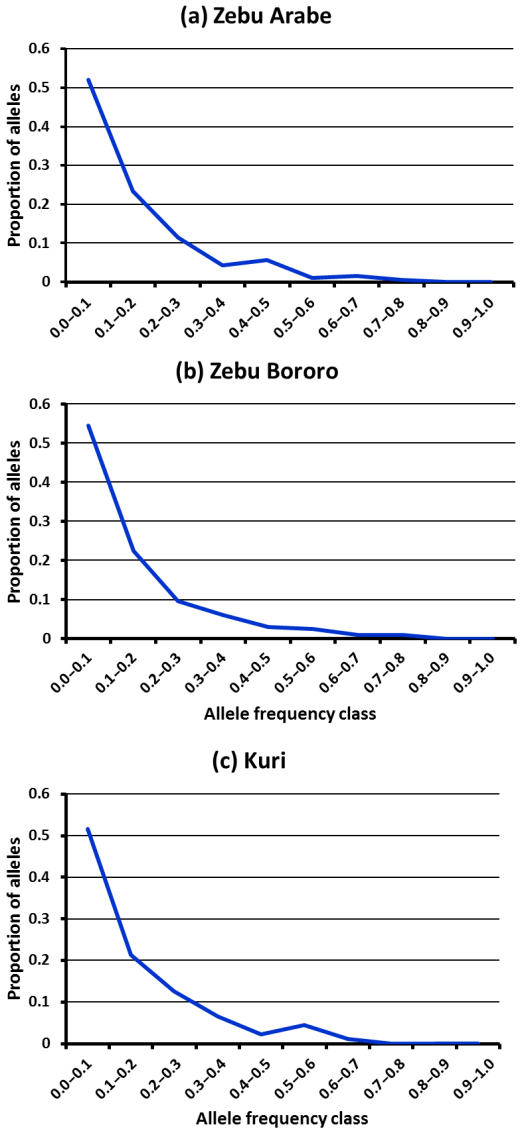


**Figure 4.** Mode shift analysis showing L-shaped distribution of allele frequencies in **(a)** Zebu Arabe, **(b)** Zebu Bororo and **(c)** Kuri breeds of Niger cattle.

## 4 Conclusion

The present study reports high genetic diversity and moderate levels of estimated inbreeding in three important breeds of Niger cattle. The global $F$ statistics showed low genetic differentiation among Niger cattle with about 2.6 % of total

variation being attributed to between-breed differences. High levels of admixture were evident from the distribution of pairwise inter-individual allele sharing distances that showed individuals across populations being more related than individuals within populations. High genetic diversity and poor genetic structure among indigenous cattle breeds of Niger could be possibly due to historic zebu–taurine admixture and ongoing breeding practices in the region. High rate of gene flow is a cause for concern, particularly in breeds like Kuri where the genetic uniqueness is being lost rapidly. The results of the present study are expected to help in formulating effective strategies for conservation and genetic improvement of indigenous Niger cattle breeds.

**Data availability.** The original data from the study will be available upon request to the corresponding author.

**Author contributions.** AT, MI and KP designed the project; MG, MH, MA, HHT and YA collected breed information and samples; MG, MH and MA performed extraction of DNA; MS and RP performed PCR and genotyping; KP, AT, MI and AS performed analysis of data and wrote the manuscript.

**Competing interests.** The authors declare that they have no conflict of interest.

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