Genetic variation of Nigerian cattle inferred from maternal and paternal genetic markers

David H Mauki [Equal first author, 1, 2, 3], Adeniyi C Adeola [Equal first author, 1, 2], Said I Ng'ang'a [1, 2, 3], Abdulfatai Tijjani [4], Akanbi I Mark [5], Oscar J Sanke [6], Abdussamad M Abdussamad [7], Sunday C Olaogun [8], Jebi Ibrahim [9], Philip M Dawuda [10], Godwin F Mangbon [11], Paul S Gwakisa [12], Ting-Ting Yin [1], Min-Sheng Peng [1, 2, 3], Ya-Ping Zhang [Corresp. 1, 2, 3, 13, 14]

1 State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China

2 Chinese Academy of Sciences, Sino-Africa Joint Research Center, Kunming, Yunnan, China

3 University of Academy of Sciences, Kunming College of Life Science, Kunming, Yunnan, China

4 School of Life Sciences, University of Nottingham, Nottingham, United Kingdom

5 Ministry of Agriculture and Rural Development, Secretariat, Ibadan, Oyo, Nigeria

6 Taraba State Ministry of Agriculture and Natural Resources, Jalingo, Taraba, Nigeria

7 Department of Animal Science, Faculty of Agriculture, Bayero University, Kano, Kano, Nigeria

8 Department of Veterinary Medicine, University of Ibadan, Ibadan, Oyo, Nigeria

9 College of veterinary medicine, department of theriogenology, University of agriculture, Makurdi, Makurdi, Benue, Nigeria

10 Department of Veterinary Surgery and Theriogenology, College of Veterinary Medicine, University of Agriculture Makurdi, Makurdi, Benue, Nigeria

11 Division of Veterinary Office, Serti, Taraba, Nigeria

12 Department of Microbiology, Parasitology and Biotechnology/ Genome Science Center, Sokoine University of Agriculture, Morogoro, Tanzania

13 State Key Laboratory for Conservation and Utilization of Bio-Resource in Yunnan, School of Life Sciences,, Yunnan University, Kunming, Yunnan, China

14 Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences, Kunming, Yunnan, China

Corresponding Author: Ya-Ping Zhang
Email address: zhangyp@mail.kiz.ac.cn

The African cattle provide unique genetic resources shaped up by both diverse tropical environmental conditions and human activities, the assessment of their genetic diversity will shade light on the mechanism of their remarkable adaptive capacities. We therefore analyzed the genetic diversity of cattle samples from Nigeria using both maternal and paternal DNA markers. Nigerian cattle can be assigned to 80 haplotypes based on the mitochondrial DNA (mtDNA) D-loop sequences and haplotype diversity was 0.985 + 0.005. The network showed two major matrilineal clustering: the dominant cluster constituting the Nigerian cattle together with other African cattle while the other clustered Eurasian cattle. Paternal analysis indicates only zebu haplogroup in Nigerian cattle with high genetic diversity 1.000 ±0.016 compared to other cattle. There was no signal of maternal genetic structure in Nigerian cattle population, which may suggest an extensive genetic intermixing within the country. The absence of *Bos indicus* maternal signal in Nigerian cattle is attributable to vulnerability bottleneck of mtDNA lineages and concordance with the view of male zebu genetic introgression in African cattle. Our study shades light on the current genetic diversity in Nigerian cattle and population history in West Africa.

1 **Genetic variation of Nigerian cattle inferred from maternal and paternal genetic markers**

3 David H Mauki [Equal first author, 1, 2, 3], Adeniyi C Adeola [Equal first author, 1, 2] , Said I Ng'ang'a [1, 2, 3],

4 Abdulfatai Tijjani [4], Akanbi I Mark [5], Oscar J Sanke [6], Abdussamad M Abdussamad[7], Sunday C

5 Olaogun[8], Jebi Ibrahim[9], Philip M Dawuda[10], Godwin F Mangbon[11], Paul S Gwakisa[12], Ting-Ting

6 Yin[1], Min-Sheng Peng [1, 2, 3], Ya-Ping Zhang[1, 2, 3, 13, 14,*]

8 [1]State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology,

9 Chinese Academy of Sciences, Kunming, Yunnan, China.

10 [2]Chinese Academy of Sciences, Sino-Africa Joint Research Center, Kunming, Yunnan, China.

11 [3]University of Academy of Sciences, Kunming College of Life Science, Kunming, Yunnan,

12 China.

13 [4]School of Life Sciences, University of Nottingham, Nottingham, United Kingdom.

14 [5]Ministry of Agriculture and Rural Development, Secretariat, Ibadan, Oyo, Nigeria.

15 [6]Taraba State Ministry of Agriculture and Natural Resources, Jalingo, Taraba, Nigeria.

16 [7]Department of Animal Science, Faculty of Agriculture, Bayero University, Kano, Nigeria.

17 [8]Department of Veterinary Medicine, University of Ibadan, Ibadan, Oyo, Nigeria.

18 [9]College of veterinary medicine, department of theriogenology, University of agriculture,

19 Makurdi, Makurdi, Benue, Nigeria.

20 [10]Department of Veterinary Surgery and Theriogenology, College of Veterinary Medicine,

21 University of Agriculture Makurdi, Makurdi, Benue, Nigeria

22 [11]Division of Veterinary Office, Serti, Taraba, Nigeria.

23 [12]Department of Microbiology, Parasitology and Biotechnology/ Genome Science Center, Sokoine

24 University of Agriculture, Morogoro, Tanzania.

25 [13]State Key Laboratory for Conservation and Utilization of Bio-Resource in Yunnan, School of

26 Life Sciences, Yunnan University, Kunming, Yunnan, China.

27 [14]Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences,

28 Kunming, Yunnan, China.

29 *Corresponding Author: Ya-Ping Zhang

30 Email address: zhangyp@mail.kiz.ac.cn

32 Abstract

33 The African cattle provide unique genetic resources shaped up by both diverse tropical

34 environmental conditions and human activities, the assessment of their genetic diversity will

35 shade light on the mechanism of their remarkable adaptive capacities. We therefore analyzed the

36 genetic diversity of cattle samples from Nigeria using both maternal and paternal DNA markers.

37 Nigerian cattle can be assigned to 80 haplotypes based on the mitochondrial DNA (mtDNA) D-

38   loop sequences and haplotype diversity was 0.985±0.005. The network showed two major

39   matrilineal clustering: the dominant cluster constituting the Nigerian cattle together with other

40   African cattle while the other clustered Eurasian cattle. Paternal analysis indicates only zebu

41   haplogroup in Nigerian cattle with high genetic diversity 1.000 ± 0.016 compared to other cattle.

42   There was no signal of maternal genetic structure in Nigerian cattle population, which may

43   suggest an extensive genetic intermixing within the country. The absence of *Bos indicus*

44   maternal signal in Nigerian cattle is attributable to vulnerability bottleneck of mtDNA lineages

45   and concordance with the view of male zebu genetic introgression in African cattle. Our study

46   shades light on the current genetic diversity in Nigerian cattle and population history in West

47   Africa.

48

49   Keywords: mtDNA, Y-chromosome, genetic diversity, Nigerian cattle, West Africa

50

51   **INTRODUCTION**

52   The modern domestic cattle were initially domesticated about 10,000 years ago from two putative

53   domestication centers, the Near East for *Bos taurus* and the Indian Sub-continent for *B. indicus*

54   (*Loftus et al., 1994a*). In Africa, the modern domestic cattle were probably introduced at different

55   times with *B. taurus* circa 7,000 – 4,000 years BP and *B. indicus* circa 4,000 – 2,000 years BP

56   from their putative centers of domestication (*Meghen, MacHugh & Bradley, 1994; Freeman et al.,*

57   *2004*). However, the zebu cattle were also reintroduced to the African continent by Arab traders

58   around ~699 - 640 years AD following the death of the Prophet (*Bradley et al., 1998*). Penetration

59   of the predominant African taurine to West Africa was amid 4,000 years BP (*Marshall &*

60 *Hildebrand, 2002*). The post-introduction of the zebu cattle led to their spread sporadically to

61 West Africa circa 1,400 years ago (*Meghen, MacHugh & Bradley, 1994; Hanotte et al., 2002)*

62 from East Africa hypothesized to be the original entry point of zebus in Africa (*Gifford-Gonzalez*

63 *& Hanotte, 2011*). To date, the origin and migration of ancient and modern African cattle is still

64 under strong debate due to conflicting archaeological and genetic evidence (*Hanotte et al., 2000,*

65 *2002; Magnavita, 2006*), therefore, unravelling of any possible clues is still at large and with very

66 crucial pressure sought-after by scientist and archaeologist.

67 In the past decade scientist have been using mtDNA markers in the control region and the entire

68 mtDNA genome to understand phylogenetic tree models for describing origin, divergence times

69 and domestications of cattle and their population expansion to different parts of the globe (*Achilli*

70 *et al., 2008; Ho et al., 2008; Horsburgh et al., 2013; Olivieri et al., 2015*). MtDNA studies on

71 cattle have grouped them in two major lineages represented by haplogroup T for *B. taurus* (*Troy et*

72 *al., 2001*) and haplogroups I1 and I2 (*Chen et al., 2010*) both defining the genetic lineage of *B.*

73 *indicus*. Within the haplogroup T, sister sub-haplogroups T1, T2, T3, T4 and T5 were revealed

74 (*Mannen et al., 2004; Achilli et al., 2008, 2009*). The haplotypes T2 and T3 are the dominant

75 haplotypes in the Middle East and Europe and also in Africa at very low frequency (*Beja-Pereira*

76 *et al., 2006; Olivieri et al., 2015*). Majority of the mtDNA haplotypes in African cattle are defined

77 by T1 haplogroup (*Troy et al., 2001; Horsburgh et al 2013*), with no evidence of zebu mtDNA

78 haplotypes been reported so far. There has been some more discoveries of other haplotypes P, Q,

79 R, E and C especially when analysis of whole mtDNA genome was applied (*Achilli et al 2008,*

80 *2009; Bonfiglio et al., 2010*), with only haplotype Q (Q1) being reported recently in the African

81 cattle (*Olivieri et al., 2015*).

82 Genetic studies on paternal Y-chromosomal DNA markers have previously reported the frequency

83 of both haplogroups Y1 and Y2 for taurines and of Y3 haplogroup an exclusive for zebus

84 (*Götherström et al., 2005; Bonfiglio et al., 2012b; Ginja et al., 2010*). Some authors like *Perez-*

85 *Pardal et al. (2010), Alvarez et al. (2017)* and *Chen et al. (2018)* have reported both Y1 and Y2

86 haplotype frequencies in sub-Saharan and Mediterranean regions in modern African cattle at

87 considerable varying frequencies. However, majority of the African cattle have been reported to

88 be more of zebu background (*Hanotte et al., 2000*) solely defined by haplogroup Y3 and are

89 distributed at much higher frequency than Y1 or Y2, enormously across Africa encompassing both

90 the North, West, East, Central and southern African regions (*Perez-Pardal et al., 2010, 2018*;

91 *Alvarez et al., 2017*; *Ginja et al., 2019*).

92 Nigeria, a country in West Africa harbors both the taurine and zebu cattle (*Rege, 1999*) and their

93 crossbreds (*Loftus et al., 1994a*). Apart from their economic benefit such as meat, milk, and skin,

94 the Nigerian cattle are observed as important idols in ceremonial rituals and also utilized in

95 drafting and ploughing during farming. Acquisition of the current knowledge regarding the genetic

96 status of Nigerian cattle is crucial for conservation and utilization of their genetic resources.

97 Previous genetic diversity studies in West African cattle, particularly in Nigeria are limited by the

98 few number of populations (*Bradley et al., 1994; Loftus et al., 1994a; Loftus et al., 1994b;*

99 *Bradley et al., 1996; Perez-Pardal et al., 2018*). This could imply that the actual extent of the

100 genetic diversity of Nigerian cattle still remains an enigma.

101 To disclose the genetic diversity in Nigerian cattle, we employed the use of both mtDNA and Y-

102 chromosomal markers, which have been widely used in assessing the diversity and

103 phylogeographic structure of many domestic animals (*Lindgren et al., 2004; Ramirez et al., 2009;*

104 *Wang et al., 2014; Alvarez et al., 2017*). In this study, we evaluated variation in the mtDNA D-

105 loop and Y-chromosome using 139 Nigerian cattle samples. Due to the nature of husbandry

106 management in most of African countries, the assessment of genetic variation in Nigerian cattle

107 was conducted based on their sampled locations such as from North western (Zamfara, Kano,

108 Katsina, Kaduna, and Sokoto States); North eastern/central (Taraba, and Plateau States) and

109 western (Oyo State) regions of Nigeria (Fig. 1a).

110 **MATERIALS AND METHODS**

111 **Ethical considerations**

112 All experimental procedures in the present study were performed in accordance to Research

113 Guidelines for the Institutional Review Board of Kunming Institute of Zoology, Chinese

114 Academy of Sciences (SMKX2017009) and the Central Abattoir, Ibadan, Ministry of

115 Agriculture and Rural Development, Oyo State, Nigeria. Several cattle are usually transported

116 from farms in different states in Nigeria to the Central Abattoir in Ibadan. Therefore, all cattle

117 samples were then collected at the Central Abattoir, Ibadan.

118 **Sampling and data collection**

119 A total of 139 Nigerian cattle individuals (119 females and 20 males) were sampled from

120 farmer's herds in eight different States in Nigeria as follows (Fig. 1a); Kaduna State (n=19

121 females; n=3 males), Kano State (n=4 females; n=2 males), Katsina State (n=4 females; n=2

122 males), Sokoto State (n=27 females; n=4 males), Mambilla plateau in Taraba State (n=35

123 females; n=2 males), Zamfara State (n=8 females; n=2 males), Oyo State (n=1 female; n=2

124 males) and Jos city in Plateau State (n=21 females; n=2 males). During sample collection,

125 genetically unrelated cattle from the eight states in Nigeria, ecological and geographical

126 perspectives were considered. This included randomly sampling of at least two animals per

127   household and only from those households located approximately 0.5 km further apart. Farmers

128   were also interviewed on the pedigree information of their animals prior to carrying out blood

129   collection. Blood samples were kept in 95% ethanol at room temperature before transportation to

130   the laboratory. Samples were stored at 4ºC for immediate use, or at -80ºC for later use.

131   Yak (*Bos grunniens*: Accession no. MN398192, *Huang et al., 2020*), was used as an outgroup in

132   phylogenetic tree analysis. A total of 420 sequences based on 636 bp (of the D-loop region) that

133   included 119 individuals from Nigeria, 76 from Europe, 16 from West Asia, and 209 from other

134   African countries (31 Egyptian cattle, 16 Mozambiquan cattle, 126 Ethiopian cattle, 34 Nguni

135   cattle from South Africa, and two additional samples from Nigeria) were used in mtDNA

136   analyses (more information in Figure 1 legend part b; Table S1a). For Y-chromosome analyses

137   only few representative samples of the three major haplogroups of cattle were used as detailed in

138   supplementary Table S2.

139   **DNA extraction, PCR and sequencing**

140   Genomic DNA was extracted from ~5ml of blood following phenol-chloroform method

141   (Sambrook & Russell, 2001). We amplified 636-base pair of the D-loop region of mtDNA, using

142   primers constructed from L27712 D-loop sequence for both forward and reverse primers (Table

143   S3) (*Loftus et al., 1994a*). The mtDNA amplification and sequencing reactions were carried out in

144   a total of 25 µl PCR reaction mixture using ~40 ng of mtDNA, 10 pmol of each primer, 2.5 mM

145   dNTPs and 5 units of Takara Taq DNA polymerase in a 10 pmol reaction buffer containing 1.5

146   mM $MgCl_2$. Amplifications were carried out in a thermocycler for at least 35 cycles as follows:

147   95ºC for 5 min, 94ºC for 45 sec, 58ºC for 30 sec, 72ºC for 1 min 30 sec, and a final extension of 7

148   min at 72ºC. The quality and confirmation check were performed using 2% agarose gel and

149   visualization under UV transilluminator.

150 We have sequenced 286 bp of the bovine Y-chromosome in 20 Nigerian cattle (Table S2 and S3)

151 in order to identify polymorphic sites in the X-degenerate male specific regions of the bovine Y-

152 chromosome (MSY) (*Chang et al., 2013*). In particular, a region of the zinc finger protein Y-

153 linked (ZFY) gene similar to that of Homo sapiens (human) intron 10 was used as previously

154 described (*Ginja, Telo da Gama & Penedo, 2009*; Table S3) for the amplification and sequencing

155 of all 20 samples at the length of 286 bp. We applied the same PCR conditions used in the

156 amplification of mtDNA with exceptional of the annealing temperature of 53 ^{o}C. The quality

157 control checks as described in mtDNA were also carried out on all the 20 PCR amplicons. The

158 amplified mtDNA and Y-chromosomal DNA fragments were subjected to sequencing procedures

159 such as purification of the PCR products with Exo - SAP-IT Cleanup kit as per manufacturer's

160 instructions (Affymetrix). Subsequently, sequencing reactions were carried out using the

161 BigDye™ Terminator v3.1 Cycle Sequence Kit (Applied Biosystems) and the generated products

162 from this step were further purified by alcohol precipitation. Lastly, we used ABI PRISM 3730

163 automated DNA sequencer (Applied Biosystems) to sequence the purified products. All

164 sequences were assembled in SeqMan Lasergene package in DNASTAR software.

165 **Data analysis**

166 **Sequences check and alignment**

167 The assembled DNA sequences of 139 individuals were exported into MEGA X ver 10.1.7

168 software (*Kumar et al., 2018*) for alignment with other cattle populations mined from Genbank

169 for both mtDNA D-loop (Table S1a and S1b) and ZFY Y-chromosomal markers (Table S2).

170 Multiple sequence alignments of the D-loop region and ZFY gene of the Y-chromosome were

171 carried out using CLUSTAL W package (*Thompson, Higgins & Gibson, 1994*) integrated in the

172   MEGA software. All sites containing alignment gaps were excluded from the analysis. Our data

173   involved amplification and sequencing of the 636 -base pair (bp) mtDNA D-loop region and 286

174   bp ZFY gene of 119 and 20 Nigerian cattle samples respectively. Variations in the D-loop region

175   were detected by assembling all forward and reverse sequences against the reference *B. taurus*

176   mtDNA sequence [(GenBank accession no. V00654); (*Anderson et al., 1982*)]. Variations in the

177   Y-chromosome of the X degenerate region located at intron 10 of ZFY gene were also

178   determined. The alignments and assembly of the Y-chromosome sequences were carried out

179   similarly following the protocol used by *Ginja, Telo da Gama & Penedo, (2009)* where

180   sequences from distantly related species were used as described by *Götherström et al. (2005)* but

181   using *Bison bison*, *Bos frontalis*, and *B. grunniens* sequences (*Verkaar et al., 2004*). The

182   polymorphic sites in the Y – chromosome male specific region were identified using a Y –

183   chromosome *B. taurus* reference genome (GenBank Accession no. AF241271; *Lawson et al.,*

184   *2002*).

185   **Genetic diversity and haplogroup classification**

186   MitoToolPy_Linux (*Peng et al., 2015*) was used to determine haplogroup distribution across

187   Africa by analyzing a 240 bp fragment length of the D-loop region of mtDNA involving 609

188   cattle samples in total (including the 119 Nigerian cattle sequenced in this study [(Fig. 1a; Table

189   S1b); (reference data were retrieved from: *Loftus et al., 1994a; Bradley et al., 1996; Troy et al.,*

190   *2001; Beja-Pereira et al., 2006; Dadi et al., 2009; Bonfiglio et al., 2012a; Horsburgh et al.,*

191   *2013; Olivieri et al., 2015*)] . We used DnaSP v5 (*Librado & Rozas, 2009*) to determine the

192   haplotypes in 420 mtDNA cattle sequences and the assignment of bovine Y-chromosome

193   haplogroups for Nigerian cattle. Genetic diversity, was assessed by using Arlequin v3.5

194   (*Excoffier & Lischer, 2010*) and expressed in terms of the total number of haplotypes (H) and

195 polymorphic sites (PS), haplotype diversity (HD), nucleotide diversity (π), the mean number of

196 nucleotide differences (Df) and their standard deviations (SD) estimated across all African

197 populations used in this study. Notably, the comparisons of the genetic diversity estimates were

198 considered for only those populations with sample size above 5.

199 **Phylogenetic tree analyses**

200 To investigate the evolutionary relationship of Nigerian cattle with other cattle samples mined

201 from GenBank (Table S1a and S2), the same version of MEGA software was used to construct a

202 rooted neighbor-joining (NJ) phylogenetic tree (*Saitou & Nei 1987*) using the Maximum

203 Composite Likelihood evolutionary distance approach *(Tamura, Nei & Kumar, 2004)* and

204 bootstrap test was employed at 1000 replications so as to assess the confidence of each node

205 (*Felsenstein, 1985*). To further visualize the genetic relationships between the haplotypes and

206 identifying the number of unique mtDNA D‐loop haplogroups present in the 420 dataset, the

207 median‐joining (MJ) network (*Bandelt, Forster & Rohl, 1999*) was generated by using the

208 default setting weights of both transversions and transitions as implemented in Network v4.6

209 software (www.fluxus-engineering.com).

210 **Population genetic structure and demographic dynamic profiles**

211 To infer the matrilineal genetic variation within populations, among populations, and groups of

212 populations, analysis of molecular variance (AMOVA) was carried out following 50,000

213 permutations in Arlequin v3.5 software. The analysis was conducted for Nigerian cattle at

214 various hierarchical levels *viz* the Nigerian cattle as a single cluster, Nigerian cattle vs the other

215 African countries but also vs cattle from Europe and West Asia. The levels of significance in

216   each hierarchical cluster tested were evaluated using F_{ST} parameter at a significant P level of

217   0.05.

218   To investigate the population dynamics and demographic patterns of Nigerian cattle population,

219   mismatch distribution patterns were estimated (*Rogers & Harpending, 1992*) with respect to

220   their geographical regions for North West and North East. The chi‑square test of goodness of

221   fit and Harpending's raggedness index "*r*" (*Harpending, 1994*) statistics were also calculated to

222   assert the significance of the deviations of the sum of squares differences (*SSD*) observed from

223   the simulated model of demographic expansions determined by 1,000 coalescent simulations.

224   Demographic statistical parameters for Tajima's D (*Tajima, 1989*) and Fu's F_S (*Fu, 1997*) were

225   also estimated by using Arlequin v3.5 software to further complement the mismatch distribution

226   patterns.

227

228   **RESULTS**

229   **MtDNA genetic diversity**

230   In this study we evaluated variations in the mtDNA D-loop of 119 Nigerian cattle together with

231   301 global cattle sequences based on 636 bp from Egypt, Ethiopia, Mozambique, South Africa,

232   Europe and West Asia available in the GenBank. The sequences generated in this study have

233   been deposited in the GenBank with accession numbers MT362777 – MT362895. There were

234   153 variable sites scored in all 420 cattle samples that defined 275 haplotypes (Table S4) and 80

235   of them assigned to Nigerian cattle sequenced in this study (Table 1). Most of the Nigerian cattle

236   in the current study possess unique haplotypes (80%) and the remaining ones were shared with

237   other African and European cattle. The lowest level of haplotype diversity (0.983 ± 0.009) was

238   observed in cattle from North East while the highest (0.984 ± 0.007) was observed in the North

239   Western region. Estimated haplotype diversity (HD) across all Nigerian individuals was

240   0.985+0.005 (Table 1). This value observed is lower than the haplotype diversity of Egyptian

241   and Mozambican cattle populations but was higher compared to Ethiopian and South African

242   cattle.

243   **Haplogroup classification and phylogenetic trees using mtDNA**

244   The haplogroup distribution across Africa shows the majority of African cattle are of *B. taurus*

245   T1 the widely known *B. taurus* haplogroup for African cattle (Fig. 1a, Table S1b). The Nigerian

246   cattle in this study were all classified into haplogroup T1 (Table S1a and S1b), and majority of

247   them constitute of haplogroup T1a similar to findings by *Olivieri et al. (2015)*. Apart from

248   Nigeria, other African countries in the Northern part of Africa particularly Egypt, Libya and

249   Morocco also carried other types of *B. taurus* haplogroups T2, T3 and Q1. No traces of *maternal*

250   lineages were observed in Nigerian cattle samples sequenced in this study.

251   To obtain further insights into the haplotype relationships, the network analysis (Fig. 1b) and

252   phylogenetic tree (Fig. S1) were constructed using the 636 bp sequences of 119 Nigerian cattle

253   and 301 other sequences retrieved from the GeneBank. The phylogenetic tree depicted two major

254   lineages of cattle, *B. taurus* (T1, T2, T3, T5, and Q1) and *B. indicus* (I1 and I2) lineages as

255   expected (*Chen et al., 2010; Achilli et al., 2008*). All Nigerian cattle have been placed together

256   with the rest of taurine cattle individuals separately from the zebu cattle. The MJ network

257   depicted similar pattern where haplogroup I1 for zebu lineage was separated from all the taurine

258   haplogroups. Furthermore, the network revealed two major clustering: the first cluster showed

259   grouping of Nigerian cattle with African and European cattle; while the second cluster did not

260   contain Nigerian individuals. Cattle samples in the first cluster exhibit a star-like pattern, a

261   matrilineal characteristic which signifies a signature of population expansion. Furthermore, we

262   found two Nigerian individuals which showed interesting results. These individuals have been

263   placed within the monophyletic clade containing individuals of haplogroups T1, T5 and Q1 from

264   Europe, which could indicate a similar pattern of origin with European cattle (*Achilli et al.,*

265   *2008*). One of the individuals (71_TAR_NIG) possess similar mutations as T5 (*Achilli et al.,*

266   *2008*) at g.16255 and 16197 (Table S5) which concurs with the phylogenetic tree results. The

267   second individual (111_KAD_NIG) appears like a basal lineage to this monophyletic clade

268   where individuals of haplogroups T5, T1 and Q1 are clustering. Our analysis also shows some

269   shared polymorphism between Nigerian cattle (71_TAR_NIG and 111_KAD_NIG), the *Bison*

270   *bison* and *B. grunniens* at transitional mutations G/A: g.15921 and T/C: g.16204 (Table S5) as

271   previously detected by (*Achilli et al., 2008*).

272   **Population genetic structure and historical demographic dynamics**

273   AMOVA analysis incorporating the eight populations from Nigeria showed that more than 99%

274   of the total genetic variation present in Nigerian cattle occurred within individuals (Table S6).

275   Furthermore, when analyzing the genetic variation between Nigerian cattle and other cattle

276   populations AMOVA showed that, 62 to 93% of the total variation between Nigerian cattle and

277   other African cattle populations occurs within individuals, with the highest variation observed

278   between Nigerian and Mozambican cattle (93.46%). Generally, all attempts to explore the

279   differentiation between Nigeria and other populations were attributable to within-population

280   variance ($P < 0.05$) with exceptional of variation among populations within groups for Nigerian

281   and European cattle (F = 0.07375, $P = 0.00004$). The F_{ST} distance values between most Nigerian

282   cattle sub-populations were low (Table S7). However, it was considerably high between Nigerian

283   cattle and other African cattle populations showing significant genetic variation between them at

284    $P < 0.05$ with exceptional for cattle populations between Ethiopia and Mozambique ($FST = $ -

285   0.00548, $P = 0.57129$). Our AMOVA results complemented by genetic distance estimates

286   suggest general absence of maternal genetic structuring in Nigerian cattle sub-populations, likely

287   due to extensive genetic intermixing within the country.

288   To elucidate the demographic dynamics of Nigerian cattle, mismatch distribution patterns, for

289   each geographical region in Nigeria were assessed (Fig. S2). The mismatch distribution patterns

290   were unimodal, however the pattern deviated significantly from expected under a null hypothesis

291   model of either spatial or demographic expansion due to significant values obtained for Sum of

292   Squared deviation (SSD) and Harpending's Raggedness index (HRI) (Table S8). The significant

293   values for SSD and HRI indicate a bad goodness of fit test, which does not support the scenario

294   of population expansion. The values for Tajima's D -2.315 ($P < 0.05$) and Fu's F_S statistics -

295   26.203 ($P < 0.05$) on the other hand were both negative and significant indicating an agreement

296   of recent population growth and expansion respectively. Cattle population from Western region

297   part of Nigeria showed Tajima's D value of 0 possibly due to only 3 individuals were sampled,

298   which might indicate that the population in that region evolved as per mutation drift equilibrium

299   with no evidence of selection.

300   **Genetic polymorphism of Y- chromosome and haplogroup distribution**

301   In supplementary Figure 3, we provide detailed information on haplogroup distribution based on

302   Y-chromosomal markers in Africa (*Perez-Pardal et al., 2018; Ginja, Telo da Gama & Penedo,*

303   *2009*). The sequences generated in this study have been deposited in the GenBank with accession

304   numbers MW284951– MW284970. Our results show that all Nigerian cattle belong to

305   haplogroup Y3 solely a *B. indicus* haplogroup due to similar mutations (Table 2, Supplementary

306   Data S1). Notably, in addition to previously reported mutations that further classify zebu cattle

307   into haplotype Y3 families as pointed out by *Chen et al. (2018)* and *Perez-Pardal et al. (2018)*,

308   we also show new mutations in Nigerian cattle possibly not previously reported. These include

309   those SNPs observed between g.784 and g.805bp (Table 2, Supplementary Data S1). Multiple

310   sequence alignment with haplotypes defining the three major haplogroups of cattle (Y1, Y2 and

311   Y3) (*Nijman et al., 2008*; *Ginja, Telo da Gama & Penedo, 2009* and Table S2) revealed the

312   existence of seven mutations including mutations A>G, T>C and T>G that distinguish Y3a (or

313   Y_C) and Y3b (or $Y3_A$, $Y3_B$) haplotype families (Table 2) grouping Nigerian cattle into zebu Y3b

314   sub-haplotype family (Supplementary Data S1) due to having similar polymorphic information at

315   sites A/A and T/T (Supplementary Data S1). Majority of the Nigerian cattle shared haplotype

316   Hap_7Y3 together with cattle from Asia (Fig. 2) which may signify possible shared origin from

317   the most recent common ancestor (TMRCA). The phylogenetic tree shows that Nigerian cattle

318   cluster separately from European Y1 or Y2 haplogroups (Fig. S4). The phylogenetic tree showed

319   some of the Nigerian cattle clustering together with *B. grunniens* (yak) and *B. bison,* these two

320   latter populations are considered distantly related populations to the major lineages of domestic

321   cattle (Fig. S4) s

322   The overall genetic diversity for Nigerian cattle measured by the haplotype diversity and the

323   mean number of pairwise difference is 1.000 ± 0.016 and 1.679 ± 1.027 respectively (Table S9).

324   The genetic diversity detected in some African cattle populations/breeds as per *Perez-Pardal et*

325   *al. (2018)* and *Ginja, Telo da Gama & Penedo, (2019)* although different Y-chromosomal

326   markers such as microsatellite markers were used, the estimates still provide reliable information

327   needed for comparison with our samples (Table S9).

328

329   **DISCUSSION**

330 In this study we examined the genetic variation across eight Nigerian cattle populations, which

331 represent samples from one country in West Africa. The results based on mtDNA D-loop revealed

332 80 haplotypes from 119 Nigerian cattle sequences, which showed a haplotype diversity of

333 0.985 ± 0.005. The haplotype diversity in Nigerian cattle is lower compared to those of Egyptian

334 cattle $H = 1.0 \pm 0.008$ (*Olivieri et al., 2015*). These findings signify a high level of maternal

335 genetic variation in Nigerian cattle. AMOVA on the other hand suggests a general absence of

336 maternal genetic structuring in Nigerian cattle that may have been as a result of extensive genetic

337 intermixing within the country. Studies show that genetic intermixing is caused by rigorous

338 transportation of domestic animals like goats, cattle, and sheep from place to place as a result of

339 their valuable resource for economic trade or cultural exchange (*Tarekegn et al., 2018*). Notably,

340 recent study has revealed the impact of African cattle pastoralism in admixture of cattle across the

341 continent, and this admixture or intermixing has been well observed in the African humped cattle

342 (*Kim et al., 2020*). It is generally known that most of the genetic variation in cattle elucidated by

343 matrilineal genetic information is attributable to geographical differences (*Hanotte et al., 2002*),

344 which can be sourced at the regional or continental levels (*Dadi et al., 2009*) rather than by their

345 morphological disparities or differences in their origins (*Alvarez et al., 2017*). We observed this

346 scenario in our study samples where the variation was explained only within populations from

347 different geographical regions in Africa such as between pairs of Nigerian cattle with other

348 African cattle populations (Table S6). On the other hand, although majority of variation can be

349 explained within a population at individual level, the variation has always been nonsignificant

350 reflecting lack of genetic structuring. This trend of unstructured matrilineal populations within

351 Africa has been reported consistently throughout the entire continent (*Alvarez et al., 2017*). One of

352 the possible reasons could have been due to the influx of a continual zebu introgression especially

353 following the great rinderpest disease that wiped out nearly 5.2 million head of cattle (*Hanotte et*

354 *al., 2000, 2002*). Nonetheless, the hybridization process between zebu and taurine which is

355 practiced throughout the continent, might somewhat explain this unstructured scenario. This is

356 usually attributed by the need of farmers to have a breed of cattle that can have both of the genetic

357 attribute including resistance to disease such as trypanosomiasis and at the same time to be

358 capable to withstand adverse tropical environmental conditions such as drought or hot or humid

359 climatic environments (*Kim et al., 2017*).

360 We also observed similar genetic diversity pattern based on Y-chromosome analysis where the

361 haplotype diversity detected was higher compared to that observed in a previous study (*Perez-*

362 *Pardal et al., 2018)* for Nigerian cattle possibly due to a single breed population sampled, albeit it

363 was similar to haplotype diversity of cattle from India and Central Asia (*Perez-Pardal et al.,*

364 *2018*). Generally, in comparison with other cattle populations in Africa, our study indicated the

365 highest genetic diversity of 1.000 ± 0.016 with the lowest in Zebu_Peul breed population from

366 Burkina Faso (Table S9). Nigerian male cattle samples have been observed with high genetic

367 diversity extrapolated by Y-chromosomal single nucleotide polymorphic marker which is

368 consistent with the scenario of the unique zebu alleles mostly found only in West Africa (*Pereze-*

369 *Pardal et al., 2010, 2018*). This was hypothesized by these authors that zebus in West Africa have

370 got unique genetic background probably because of genetic contribution from local ancient

371 humped cattle. However, even though recent zebu introgression could have occurred into this

372 region, may have not contributed much to the genetic affinity with other zebus from elsewhere

373 possibly because the introgressions were female mediated (*Hanotte et al., 2000)* which can also be

374 described in the case of Kuri cattle samples of Lake Chad (*Meghen et al., 2000)* or perhaps the

375 restocking of African zebu sires from East to West during the early 20th century was relatively low

376   (*Alvarez et al., 2017*). The high genetic divergence between zebu of West Africa and those from

377   Asia was recently unveiled by *Pereze-Pardal et al. (2018)* which further strengthens this

378   speculative hypothesis.

379   The present-day zebu-like cattle in West Africa were products of crossbreeding events between

380   the West African taurine cattle such as N'Dama and imported zebu cattle directly from Asia

381   (*MacHugh et al. 1997; Kim et al., 2017*) or rather indirectly with zebu from East Africa. We

382   computed the genetic relationships among Nigerian cattle by using MJ network (Fig. 1b) and NJ

383   tree (Fig. S1). We observed all Nigerian cattle to be exclusively of taurine T1, an African specific

384   haplogroup, and morphological resemblance of *B. indicus* such as the presence of the humps. This

385   scenario is in accordance with similar observations by *Loftus et al. (1994a)* but also a similar case

386   was depicted in zebu breeds of the Americas where they were typically found with mitochondrial

387   taurine haplogroup in their genomes (Ginja *et al.,* 2019). These observations further explain that

388   zebu introgression is typically male-mediated and that this scenario took place in many places

389   around the globe. In the light of this argument with respect to Nigerian cattle in West Africa, most

390   farmers in this region preferred zebu of male lineage due to their massive muscle size but also

391   being resistant to rinderpest that taurines are not, even though them being less vigorous towards

392   trypanosomiasis, a disease prevalent in tsetse regions of both West and Central Africa whereby

393   taurine cattle depict resistance to the disease (*Grigson, 1991; Ibeagha-Awemu et al., 2004*) The

394   complete absence of Asian zebu mtDNA in Nigerian cattle samples suggests that crossbreeding

395   events were mainly through the imported Asian male zebus (*Loftus et al., 1994a*; *Loftus et al.,*

396   *1994b*; *Bradley et al., 1996*). Nonetheless, this scenario could have been attributed by the

397   continued adoption of trypanotolerant breeds that probably led to total loss of the *B. indicus*

398   mtDNA lineages or its vulnerability towards population bottleneck. This has also been observed

399 elsewhere by several studies particularly cattle from North-East Asia (*Mannen et al., 2004*).

400 *Bradley et al. (1994)* and *Perez-Pardal et al. (2018)* further stress this observation extrapolating

401 the importation of probably only male zebus into West African region. Other factors such as

402 droughts and the great rinderpest disease outbreak or unbiased selection of cattle breeds over zebu

403 mitochondria may have contributed to the loss of any rare zebu mtDNA (*Dadi et al., 2009*).

404 We have also analyzed the Nigerian cattle using a single Y-chromosomal polymorphic marker in

405 the X-degenerate region of the male specific Y-chromosome region. Our findings based on

406 mtDNA and Y-chromosome analyses conducted in this study have confirmed that Nigerian cattle

407 are an influence of both taurine and zebu lineages from female and male genetic contributions

408 respectively, with only zebu male specific haplotypes being detected. This illustrates a complex

409 scenario of the genetic background in most of the present-day African cattle populations (*Alvarez*

410 *et al., 2017*). Previously, mtDNA studies (*Loftus et al., 1994a*) found that African cattle are of

411 taurine background. However, earlier studies conducted using Y-chromosomal markers (*Bradley*

412 *et al., 1994*) found that African cattle had been genetically introgressed with zebu cattle from Asia.

413 The introgressions were male mediated (*Hanotte et al., 2000*) and that majority of African cattle

414 belong to the zebu specific haplogroup Y3. Patrilineal studies show that zebu cattle are distributed

415 at varying frequency across the continent with more frequency observed in eastern and Central

416 Africa but at a lower frequency in the western and southern parts of Africa (Fig. S3). This

417 decrease in frequency of zebu haplogroup Y3 as one moves towards West and South of Africa is

418 possibly due to the presence of *B. taurus* European haplogroups Y1 and Y2 in these regions (Fig.

419 S3). A study by *Decker et al. (2014)* had previously demonstrated similar observations using

420 autosomal single nucleotide polymorphisms (SNPs) markers. This unevenly distribution of zebu

421 allele and introgression in the African continent is possibly attributed to selection of breeds by

422 farmers based on disease tolerance such as tolerance to trypanosomiasis or ability to withstand

423 adverse environmental conditions such as drought (*Hanotte et al., 2000*). Although, some of the

424 previous studies conducted in West African cattle detected European Y1/Y2 haplogroups (*Perez-*

425 *Pardal et al., 2010; Ginja et al., 2019*), it is surprisingly enough that our study samples from

426 Nigeria did not yield any of these, but rather showed that all Nigerian cattle are of zebu Y3 origin.

427 We believe that, the undetected Y1 or Y2 haplotypes in Nigerian cattle might possibly indicate

428 low or no influence of European cattle in these studied samples, coinciding with what was

429 reported previously by *Hanotte et al. (2000)* of 0% Y- chromosomal taurine alleles in Nigerian

430 cattle. Nonetheless, it's probable that our sampling coverage did not cover enough samples from

431 western part of Nigeria where most cattle of haplotype Y1 and Y2 are likely to be found.

432 Similarly, this was shown in phylogenetic tree (Fig. S4), where Nigerian cattle somehow clustered

433 separately from European Y1/Y2 haplotypes. Some studies based on additional Y-chromosomal

434 markers, including STRs, have further indicated that *B. indicus* haplogroup Y3 is composed of

435 three sub-haplotype families which include $Y3_A$, $Y3_B$ (also defined as Y3b nomenclature), and

436 $Y3_C$ (or Y3a) (*Chen et al., 2018; Perez-Pardal et al., 2018*), with $Y3_A$ being a cosmopolitan

437 haplotype, while $Y3_B$ is exclusively found in West African cattle. These three haplotypic zebu

438 families co-existed together with their counterpart taurine in approximately 200,000 years ago

439 (*Loftus et al., 1994a; Ho et al., 2008; Murray et al., 2010*). However, they diverged at different

440 times among themselves before the start of domestication, with the most recent divergence

441 occurring between West and East African zebus (*Perez-Pardal et al., 2018*).

442 Moreover, our study observed that the Nigerian cattle of Y3 haplogroup can be further classified

443 into sub-haplotype family Y3b based on the nomenclature adopted by *Chen et al. (2018)*

444 (Supplementary Data S1) with several zebu strains (Fig. 2) which possibly reflect less intensive

445 selection in African countries as observed in Nigeria compared to other regions for instance

446 Europe (Xu et al 2015). Our findings indicate a possibility of gene flow or backcrossing of cattle

447 with wild stock from other related bovine species which may have had occurred probably during

448 initial stages of early domestication in the Eurasia prior to population expansion or at later

449 instances post domestication around 2000 years ago (*Chen et al., 2010; Perez-Pardal et al., 2018*).

450 These possible imprints of gene flow or introgression with wild stock or other related domestic

451 Bovidae was inferred through the shared matrilineal mutation motifs in the wild bison (*Bison*

452 *bison*), the domestic yak (*B. grunniens*) and Nigerian cattle. This was clearly confirmed by the

453 patrilineal phylogeny where some of the Nigerian cattle clustered closely with yak, the *B.*

454 *grunniens* and *B. bison* (Fig. S4). Our finding is coherent with a study by *Chen et al. (2018)*,

455 where they were able to detect evidence of gene flow and adaptive introgression between Chinese

456 zebu cattle and the Banteng (*B. javanicus*). Nonetheless, previous studies have also reported

457 possibility of secondary introgression events that might have occurred from the wild oxen stock

458 into the gene pool of domestic cattle during early domestication (*Achilli et al., 2008, 2009*). The

459 observed shared wild stock mutations in Nigerian cattle agrees with previous studies (*Perez-*

460 *Pardal et al., 2010, 2018)* that the $Y3_B$, also termed Y3b (by *Chen et al.,* 2018) zebu lineage in

461 West Africa were probably intermingled with ancient humped cattle which would currently be a

462 significant representative reservoir of male zebu biodiversity. The legacy of the possibility of

463 subsequent introgression from local ancient wild cattle partly explains the existing large

464 divergence between West African zebus and zebus from Asia (*Perez-Pardal et al., 20 10, 2018*).

465 These observations maybe speculative, and could indicate a much more complex scenario of the

466 origin of cattle that possibly involved multiple ancestral domestication populations (*Perez-Pardal*

467 *et al., 2018*) especially when considering the discrete contribution of wild ox into the gene pools

468 of the major descendant lineages of cattle (Murray et al., 2010) or perhaps the remnant traces left

469 before the divergence between domestic *Bos* species and the ancient wild ox had taken place some

470 million years ago (*Buntjer et al., 2002*).

471

472 **CONCLUSIONS**
473 This study reported the current genetic status and some possible new insights about the origin of

474 cattle in West Africa using Nigerian samples from matrilineal and patrilineal perspectives. High

475 level of maternal and paternal genetic diversity was observed in Nigerian cattle, with lack of

476 phylogeographic structure possibly due to human mediated interventions that usually enhance

477 severe intermixing as a result of improper husbandry management practices. The phylogenetic

478 tree based on patrilineal analysis and the matrilineal haplogroup classification have both

479 provided consensus evidence of a possible introgression and gene flow from wild ancient stock

480 and other related bovine species into Nigerian cattle. We recommend carrying out in-depth

481 population genetic studies using high-throughput technologies on complete mtDNA and

482 autosomal genomes of Nigerian cattle in West Africa as well as the use of other Y-chromosomal

483 markers in order to generate comprehensive genetic information on their adaptive traits, selection

484 and demographic history.

485

486 **Authors' contributions**

487 Y.-P.Z., D.H.M, A.C.A. and M.-S.P. led the project, and designed and conceived the study.

488 D.H.M. and A.C.A. prepared the manuscript. M.-S.P. and Y.-P.Z. revised the manuscript.

489 D.H.M., S.I.N. and A.C.A performed the data analysis. A.C.A., A.I.M., O.J.S., S.C.O., J.I. and

490 G.F.M. performed sampling. D.H.M. carried out experiments. All authors revised and approved

491 the final manuscript.

492

493 **Acknowledgments**

494 We appreciate all those who assisted in the study. This work was supported by the Sino-Africa

495 Joint Research Center, Chinese Academy of Sciences (SAJC201611) and the Animal Branch of

496 the Germplasm Bank of Wild Species, Chinese Academy of Sciences (the Large Research

497 Infrastructure Funding). The Chinese Academy of Sciences President's International Fellowship

498 Initiative provided support to Adeniyi Charles Adeola (2018FYB0003). David Heriel Mauki and

499 Said Ismael Ng'ang'a acknowledge the support of the Chinese Academy of Sciences-The World

500 Academy of Sciences (CAS-TWAS) President's Fellowship Program for Doctoral students.

501

502 **Competing interests**

503 The authors declare that they have no competing interests.

504

505 **References**

506 Achilli A, Bonfiglio S, Olivieri A, Malusa A, Pala M, Hooshiar Kashani B, Perego UA, Ajmone-

507   Marsan P, Liotta L, Semino O, Bandelt HJ, Ferretti L, and Torroni A. 2009. The

508   multifaceted origin of taurine cattle reflected by the mitochondrial genome. *PLoS ONE*

509   4(6). e5753. 10.1371/journal.pone.0005753

510 Achilli A, Olivieri A, Pellecchia M, Uboldi C, Colli L, Al-Zahery N, Accetturo M, Pala M,

511   Hooshiar Kashani B, Perego UA, Battaglia V, Fornarino S, Kalamati J, Houshmand M,

512   Negrini R, Semino O, Richards M, Macaulay V, Ferretti L, Bandelt HJ, Ajmone-Marsan

513   P, and Torroni A. 2008. Mitochondrial genomes of extinct aurochs survive in domestic

514   cattle. *Current Biology* **18**(4):R157-158. 10.1016/j.cub.2008.01.019

515 Álvarez I, Pérez‑Pardal L, Traoré A, Koudandé DO, Fernández I, Soudré A, and Goyache F.

516   2017. Differences in genetic structure assessed using Y‑chromosome and mitochondrial

517   DNA markers do not shape the contributions to diversity in African sires. *Journal of*

518   *Animal Breeding and Genetics* **134**(5):393-404.

519 Anderson S, de Bruijn MH, Coulson AR, Eperon IC, Sanger F, and Young IG. 1982. Complete

520   sequence of bovine mitochondrial DNA. Conserved features of the mammalian

521   mitochondrial genome. *Journal of molecular biology* **156**(4):683-717. 10.1016/0022-

522   2836(82)90137-1

523 Bandelt HJ, Forster P, and Rohl A. 1999. Median-joining networks for inferring intraspecific

524   phylogenies. *Molecular   Biology   and   Evolution*   **16**:37-48.

525   10.1093/oxfordjournals.molbev.a026036

526 Beja-Pereira A, Caramelli D, Lalueza-Fox C, Vernesi C, Ferrand N, Casoli A, Goyache F, Royo

527   LJ, Conti S, Lari M, Martini A, Ouragh L, Magid A, Atash A, Zsolnai A, Boscato P,

528   Triantaphylidis C, Ploumi K, Sineo L, Mallegni F, Taberlet P, Erhardt G, Sampietro L,

529   Bertranpetit J, Barbujani G, Luikart G, and Bertorelle G. 2006. The origin of European

530   cattle: evidence from modern and ancient DNA. *Proceedings of the National Academy of*

531   *Sciences of the United States of America* **103**(21):8113-8118. 10.1073/pnas.0509210103

532   Bonfiglio S, Achilli A, Olivieri A, Negrini R, Colli L, Liotta L, Ajmone-Marsan P, Torroni A, and

533   Ferretti L. 2010. The enigmatic origin of bovine mtDNA haplogroup R: sporadic

534   interbreeding or an independent event of Bos primigenius domestication in Italy? *PLoS*

535   *ONE* **5**(12). e15760. 10.1371/journal.pone.0015760

536   Bonfiglio S, De Gaetano A, Tesfaye K, Grugni V, Semino O, Ferretti L. 2012b. A novel USP9Y

537   polymorphism allowing a rapid and unambiguous classification of Bos taurus Y

538   chromosomes into haplogroups. *Animal Genetics* **43**(5): 611-613.

539   Bonfiglio S, Ginja C, De Gaetano A, Achilli A, Olivieri A, Colli L, Tesfaye K, Agha SH, Gama

540   LT, Cattonaro F, Penedo MC, Ajmone-Marsan P, Torroni A, and Ferretti L. 2012a. Origin

541   and spread of Bos taurus: new clues from mitochondrial genomes belonging to haplogroup

542   T1. *PLoS One* **7**(6). e38601. 10.1371/journal.pone.0038601

543   Bradley DG, Loftus RT, Cunningham P, and MacHugh DE. 1998. Genetics and domestic cattle

544   origins. *Evolutionary Anthropology: Issues, News, and Reviews* **6**:79-86.

545   10.1002/(sici)1520-6505(1998)6:3<79::aid-evan2>3.0.co;2-r

546   Bradley DG, MacHugh DE, Cunningham P, and Loftus RT. 1996. Mitochondrial diversity and the

547   origins of African and European cattle. *Proceedings of the National Academy of Sciences*

548   *of the United States of America* **93**:5131-5135. 10.1073/pnas.93.10.5131

549   Bradley DG, MacHugh DE, Loftus RT, Sow RS, Hoste CH, and Cunningham EP. 1994. Zebu-

550   taurine variation in Y chromosomal DNA: a sensitive assay for genetic introgression in

551   west African trypanotolerant cattle populations. *Animal Genetics* **25**:7-12.

552 Buntjer JB, Otsen M, Nijman IJ, Kuiper MT, and Lenstra JA. 2002. Phylogeny of bovine species

553   based on AFLP fingerprinting. *Heredity* **88**(1):46-51. 10.1038/sj.hdy.6800007

554 Chang TC, Yang Y, Retzel EF, and Liu WS. 2013. Male-specific region of the bovine Y

555   chromosome is gene rich with a high transcriptomic activity in testis development.

556   *Proceedings of the National Academy of Sciences of the United States of America*

557   **110**:12373-12378. 10.1073/pnas.1221104110

558 Chen N, Cai Y, Chen Q, Li R, Wang K, Huang Y, Hu S, Huang S, Zhang H, Zheng Z, Song W,

559   Ma Z, Ma Y, Dang R, Zhang Z, Xu L, Jia Y, Liu S, Yue X, Deng W, Zhang X, Sun Z, Lan

560   X, Han J, Chen H, Bradley DG, Jiang Y, and Lei C. 2018. Whole-genome resequencing

561   reveals world-wide ancestry and adaptive introgression events of domesticated cattle in

562   East Asia. *Nature Communications* **9**(1):2337. 10.1038/s41467-018-04737-0

563 Chen S, Lin BZ, Baig M, Mitra B, Lopes RJ, Santos AM, Magee DA, Azevedo M, Tarroso P,

564   Sasazaki S, Ostrowski S, Mahgoub O, Chaudhuri TK, Zhang YP, Costa V, Royo LJ,

565   Goyache F, Luikart G, Boivin N, Fuller DQ, Mannen H, Bradley DG, and Beja-Pereira A.

566   2010. Zebu cattle are an exclusive legacy of the South Asia neolithic. *Mol Biology and*

567   *Evolution* **27**(1):1-6. 10.1093/molbev/msp213

568 Dadi H, Tibbo M, Takahashi Y, Nomura K, Hanada H, and Amano T. 2009. Variation in

569   mitochondrial DNA and maternal genetic ancestry of Ethiopian cattle populations. *Animal*

570   *Genetics* **40**(4):556-559. 10.1111/j.1365-2052.2009.01866.x

571 Decker JE, McKay SD, Rolf MM, Kim J, Molina Alcala A, Sonstegard TS, Hanotte O,

572   Gotherstrom A, Seabury CM, Praharani L, Babar ME, Correia de Almeida Regitano L,

573   Yildiz MA, Heaton MP, Liu WS, Lei CZ, Reecy JM, Saif-Ur-Rehman M, Schnabel RD,

574   and Taylor JF. 2014. Worldwide patterns of ancestry, divergence, and admixture in

575   domesticated cattle. *PLoS Genetics* **10**(3). e1004254. 10.1371/journal.pgen.1004254

576 Excoffier L, and Lischer HE. 2010. Arlequin suite ver 3.5: a new series of programs to perform

577   population genetics analyses under Linux and Windows. *Molecular Ecology Resources*

578   **10**:564-567. 10.1111/j.1755-0998.2010.02847.x

579 Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*

580   **39**:783-791.

581 Freeman AR, Meghen CM, MacHugh DE, Loftus RT, Achukwi MD, Bado A, Sauveroche B, and

582   Bradley DG. 2004. Admixture and diversity in West African cattle populations. *Molecular*

583   *Ecology* **13**:3477-3487. 10.1111/j.1365-294X.2004.02311.x

584 Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking

585   and background selection. *Genetics* **147**:915-925.

586 Gifford-Gonzalez D, and Hanotte O. 2011. Domesticating Animals in Africa: Implications of

587   Genetic and Archaeological Findings. *Journal of World Prehistory* **24**:1-23.

588   10.1007/s10963-010-9042-2

589 Ginja C, Gama LT, Cortes O, Burriel IM, Vega-Pla JL, Penedo C, Sponenberg P, Canon J, Sanz

590   A, do Egito AA, Alvarez LA, Giovambattista G, Agha S, Rogberg-Munoz A, Lara MAC,

591   BioBovis C, Delgado JV, and Martinez A. 2019. The genetic ancestry of American Creole

592   cattle inferred from uniparental and autosomal genetic markers. *Scientific Reports*

593   **9**(1):11486. 10.1038/s41598-019-47636-0

594 Ginja C, Penedo MCT, Melucci L, Quiroz J, Martinez Lopez OR, Revidatti MA, Gama LT. 2010.

595   Origins and genetic diversity of New World Creole cattle: inferences from mitochondrial

596   and Y chromosome polymorphisms. *Animal genetics* **41**(2): 128-141.

597 Ginja C, Telo da Gama L, and Penedo MC. 2009. Y chromosome haplotype analysis in Portuguese

598   cattle breeds using SNPs and STRs. *Journal of Heredity* **100**(2):148-157.

599   10.1093/jhered/esn080

600 Götherström A, Anderung C, Hellborg L, Elburg R, Smith C, Bradley DG, and Ellegren H. 2005.

601   Cattle domestication in the Near East was followed by hybridization with aurochs bulls in

602   Europe. *Proceedings of the Royal Society B: Biological Sciences* **272**(1579):2345-2350.

603   10.1098/rspb.2005.3243

604 Grigson C. 1991. An African Origin for African Cattle? Some Archaeological Evidence. *The*

605   *African Archaeological Review* **9**:119-144.

606 Hanotte O, Bradley DG, Ochieng JW, Verjee Y, Hill EW, and Rege JE. 2002. African pastoralism:

607   genetic imprints of origins and migrations. *Science* **296**(5566):336-339.

608   10.1126/science.1069878

609 Hanotte O, Tawah CL, Bradley DG, Okomo M, Verjee Y, Ochieng J, and Rege JRO. 2000.

610   Geographic distribution and frequency of a taurine Bostaurus and an indicine Bos indicus

611   Y specific allele amongst sub-Saharan African cattle breeds. *Molecular Ecology* **9**(4):387–

612   396.

613 Harpending HC. 1994. Signature of ancient population growth in a low-resolution mitochondrial

614   DNA mismatch distribution. *Human Biology* **66**:591-600.

615 Hiendleder S, Lewalski H, and Janke A. 2008. Complete mitochondrial genomes of Bos taurus

616   and Bos indicus provide new insights into intra-species variation, taxonomy and

617   domestication. *Cytogenetic and Genome Research* **120**(1-2):150-156.

618 Horsburgh KA, Prost S, Gosling A, Stanton JA, Rand C, and Matisoo-Smith EA. 2013. The genetic

619   diversity of the Nguni breed of African Cattle (Bos spp.): complete mitochondrial genomes

620   of haplogroup T1. *PLoS One* **8**(8):e71956. 10.1371/journal.pone.0071956

621 Huang C, Zhang Q, Fu D, Basang W, Chu M, Yangla D, Wu X, Ma X, Guo X, Yan P, and Liang

622   C. 2020. The complete mitochondrial genome sequence and phylogenetic analysis of Sibu

623   yak (*Bos grunniens*). *Mitochondrial DNA Part B* 5:46-47.

624 Ibeagha-Awemu EM, Jann OC, Weimann C, and Erhardt G. 2004. Genetic diversity, introgression

625   and relationships among West/Central African cattle breeds. *Genetics Selection Evolution*

626   **36**(6):673-690. 10.1051/gse:2004024

627 Kim J, Hanotte O, Mwai OA, Dessie T, Bashir S, Diallo B, Agaba M, Kim K, Kwak W, Sung S,

628   Seo M, Jeong H, Kwon T, Taye M, Song KD, Lim D, Cho S, Lee HJ, Yoon D, Oh SJ,

629   Kemp S, Lee HK, and Kim H. 2017. The genome landscape of indigenous African cattle.

630   *Genome Biology* **18**(1): 1-14. 10.1186/s13059-017-1153-y

631 Kim K, Kwon T, Dessie T, Yoo D, Mwai OA, Jang J, Sung S, Lee S, Salim B, Jung J, Jeong H,

632   Tarekegn GM, Tijjani A, Lim D, Cho S, Oh SJ, Lee H, Kim J, Jeong C, Kemp S, Hanotte

633   O, Kim H. 2020.The mosaic genome of indigenous African cattle as a unique genetic

634   resource for African pastoralism. *Nature Genetics* 1-12. https://doi.org/10.1038/s41588-

635   020-0694-2Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular

636   Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and*

637   *Evolution* **35**:1547-1549.

638   Lai SJ, Liu YP, Liu YX, Li XW, Yao YG. 2006. Genetic diversity and origin of Chinese cattle

639   revealed by mtDNA D-loop sequence variation. *Molecular Phylogenetics and*

640   *Evolution* **38**(1):146-154.

641   Lawson LJ, and Hewitt GM. 2002. Comparison of substitution rates in ZFX and ZFY introns of

642   sheep and goat related species supports the hypothesis of male-biased mutation rates.

643   *Journal of Molecular Evolution* **54**(1):54-61. 10.1007/s00239-001-0017-x

644   Librado P, and Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA

645   polymorphism data. *Bioinformatics* **25**:1451-1452. 10.1093/bioinformatics/btp187

646   Lindgren G, Backstrom N, Swinburne J, Hellborg L, Einarsson A, Sandberg K, Cothran G, Vila

647   C, Binns M, and Ellegren H. 2004. Limited number of patrilines in horse domestication.

648   *Nature genetics* **36**(4):335-336. 10.1038/ng1326

649   Loftus RT, MacHugh DE, Bradley DG, Sharp PM, and Cunningham P. 1994a. Evidence for two

650   independent domestications of cattle. *Proceedings of the National Academy of Sciences of*

651   *the United States of America* **91**:2757-2761. 10.1073/pnas.91.7.2757

652   Loftus RT, MacHugh DE, Ngere LO, Balain DS, Badi AM, Bradley DG, and Cunningham EP.

653   1994b. Mitochondrial genetic variation in European, African and Indian cattle populations.

654   *Anim Genetics* **25**:265-271. 10.1111/j.1365-2052.1994.tb00203.x

655   MacHugh DE, Shriver MD, Loftus RT, Cunningham P, and Bradley DG. 1997. Microsatellite

656 DNA variation and the evolution, domestication and phylogeography of taurine and zebu

657 cattle (*Bos taurus* and *Bos indicus*). *Genetics* **146**:1071-1086.

658 Magnavita C. 2006. Ancient humped cattle in Africa: a view from the Chad Basin. *African*

659 *Archaeological Review* **23**(3-4): 55-84.

660 Mannen H, Kohno M, Nagata Y, Tsuji S, Bradley DG, Yeo JS, Nyamsamba D, Zagdsuren Y,

661 Yokohama M, Nomura K, and Amano T. 2004. Independent mitochondrial origin and

662 historical genetic differentiation in North Eastern Asian cattle. *Molecular Phylogenetics*

663 *and Evolution* **32**:539-544. 10.1016/j.ympev.2004.01.010

664 Marshall F, and Hildebrand E. 2002. Cattle before crops: the beginnings of Food production in

665 Africa. *Journal of World Prehistory* **16**:99-143.

666 Meghen C, MacHugh DE, and Bradley DG. 1994. Genetic characteristics of West African cattle.

667 *World Animal Review* **78**:59-66.

668 Meghen C, MacHugh D, Sauveroche B, Kana G, Bradley D. 2000. Characterization of the Kuri

669 cattle of Lake Chad using molecular genetic techniques. The origins and development of

670 African Livestock: *Archaeology, genetics, linguistics and ethnography* 259-268.

671 Murray C, Huerta-Sanchez E, Casey F, and Bradley DG. 2010. Cattle demographic history

672 modelled from autosomal sequence variation. *Philosophical Transactions of the Royal*

673 *Society B: Biological Sciences* **365**(1552):2531-2539. 10.1098/rstb.2010.0103

674 Nijman IJ, Van Boxtel DC, Van Cann LM, Marnoch Y, Cuppen E, and Lenstra JA. 2008.

675 Phylogeny of Y chromosomes from bovine species. *Cladistics* **24**(5):723-726.

676 Olivieri A, Gandini F, Achilli A, Fichera A, Rizzi E, Bonfiglio S, Battaglia V, Brandini S, De

677   Gaetano A, El-Beltagi A, Lancioni H, Agha S, Semino O, Ferretti L, and Torroni A. 2015.

678   Mitogenomes from Egyptian Cattle Breeds: New Clues on the Origin of Haplogroup Q and

679   the Early Spread of Bos taurus from the Near East. *PLoS ONE* **10**(10):e0141170.

680   10.1371/journal.pone.0141170

681 Peng MS, Fan L, Shi NN, Ning T, Yao YG, Murphy RW, Wang WZ, and Zhang YP. 2015.

682   DomeTree: a canonical toolkit for mitochondrial DNA analyses in domesticated animals.

683   *Molecular Ecology Resources* **15**:1238-1242. 10.1111/1755-0998.12386

684 Perez-Pardal L, Royo LJ, Beja-Pereira A, Chen S, Cantet RJ, Traore A, Curik I, Solkner J, Bozzi

685   R, Fernandez I, Alvarez I, Gutierrez JP, Gomez E, Ponce de Leon FA, and Goyache F.

686   2010. Multiple paternal origins of domestic cattle revealed by Y-specific interspersed

687   multilocus microsatellites. *Heredity* **105**(6):511-519. 10.1038/hdy.2010.30

688 Perez-Pardal L, Sanchez-Gracia A, Alvarez I, Traore A, Ferraz JBS, Fernandez I, Costa V, Chen

689   S, Tapio M, Cantet RJC, Patel A, Meadow RH, Marshall FB, Beja-Pereira A, and Goyache

690   F. 2018. Legacies of domestication, trade and herder mobility shape extant male zebu cattle

691   diversity in South Asia and Africa. *Scientific Reports* **8**(1):18027. 10.1038/s41598-018-

692   36444-7

693 Ramirez O, Ojeda A, Tomas A, Gallardo D, Huang LS, Folch JM, Clop A, Sanchez A, Badaoui

694   B, Hanotte O, Galman-Omitogun O, Makuza SM, Soto H, Cadillo J, Kelly L, Cho IC,

695   Yeghoyan S, Perez-Enciso M, and Amills M. 2009. Integrating Y-chromosome,

696   mitochondrial, and autosomal data to analyze the origin of pig breeds. *Molecular Biology*

697   *and Evolution* **26**(9):2061-2072. 10.1093/molbev/msp118

698 Rege JEO. 1999. The state of African cattle genetic resources I. Classification framework and

699    identification of threatened and extinct breeds. *FAO/UNEP Animal Genetic Resources*

700    *Information Bulletin* **25**:1-25.

701 Rogers AR, and Harpending H. 1992. Population growth makes waves in the distribution of

702    pairwise genetic differences. *Molecular Biology and Evolution* **9**:552-569.

703    10.1093/oxfordjournals.molbev.a040727

704 Saitou N, and Nei M. 1987. The neighbor-joining method: a new method for reconstructing

705    phylogenetic trees. *Molecular Biology and Evolution* **4**(4):406-425.

706    10.1093/oxfordjournals.molbev.a040454

707 Sambrook J, and Russell DW. 2001. Molecular cloning. In: Laboratory CSH, ed. *A laboratory*

708    *manual*. 3 ed. New York: Cold Spring Harbor Laboratory Press.

709 Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA

710    polymorphism. *Genetics* **123**:585-595.

711 Tamura K, Nei M, Kumar S. 2004. Prospects for inferring very large phylogenies by using the

712    neighbor-joining method. *Proceedings of the National Academy of Sciences* **101**:11030-

713    11035.

714 Tarekegn GM, Tesfaye K, Mwai OA, Djikeng A, Dessie T, Birungi J, Osama S, Zergaw N, Alemu

715    A, Achieng G, Tutah J, Mutai C, Njuguna J, and Mwacharo JM. 2018. Mitochondrial DNA

716    variation reveals maternal origins and demographic dynamics of Ethiopian indigenous

717    goats. *Ecology and Evolution* **8**(3):1543-1553. 10.1002/ece3.3710

718 Thompson JD, Higgins DG, and Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of

719   progressive multiple sequence alignment through sequence weighting, position-specific

720   gap penalties and weight matrix choice. *Nucleic Acids Research* **22**(22):4673-4680.

721   10.1093/nar/22.22.4673

722 Troy CS, MacHugh DE, Bailey JF, Magee DA, Loftus RT, Cunningham P, Chamberlain AT,

723   Sykes BC, and Bradley DG. 2001. Genetic evidence for Near-Eastern origins of European

724   cattle. *Nature* **410**:1088-1091. 10.1038/35074088

725 Verkaar EL, Nijman IJ, Beeke M, Hanekamp E, and Lenstra JA. 2004. Maternal and paternal

726   lineages in cross-breeding bovine species. Has wisent a hybrid origin? *Molecular Biology*

727   *and Evolution* **21** (7):1165-1170. 10.1093/molbev/msh064

728 Wang GD, Xie HB, Peng MS, Irwin D, and Zhang YP. 2014. Domestication genomics: evidence

729   from animals. *Annual Review of Animal Biosciences* **2**:65-84.

730   Xu L, Bickhart DM, Cole JB, Schroeder SG, Song J, Tassell CP, Sonstegard TS, Liu GE. 2015.

731   Genomic signatures reveal new evidences for selection of important traits in domestic

732   cattle. *Molecular biology and evolution* **32**(3):711-725.

733   https://doi.org/10.1093/molbev/msu333

734

735 **Figure legend**

736 **Figure 1 Sampling locations of cattle in Nigeria and the network of 420 cattle samples**

737 **based on 636 bp of the mtDNA D-loop region.** (a) Map of cattle sampling locations in Nigeria

738 (Zamfara, Kano, Katsina, Kaduna, and Sokoto from North West; Taraba and Plateau from North

739   East/Central; and Oyo from the West) and the haplogroup distribution based on mtDNA across

740   Africa. The maps of Africa and Nigeria were generated by an online version of the SmartDraw

741   2012 software (https://cloud.smartdraw.com/). (b) Median-joining network of 420 cattle samples

742   constructed by using NETWORK v 4.6 (*Bandelt, Forster & Rohl, 1999*). Reference sequences

743   used for haplotype network analysis included: Europe, n=76 (*Loftus et al., 1994a; Lai et al.,*

744   *2006; Achilli et al., 2008; Hiendleder, Lewalski & Janke, 2008; Bonfiglio et al., 2012a* and

745   AF034438-AF034446 were retrieved from the GenBank); West Asia, n=16 (*Achilli et al., 2008*);

746   Egypt, n=31 (*Bonfiglio et al., 2012a; Olivieri et al., 2015*); Ethiopia, n=126 (*Dadi et al., 2009;*

747   *Bonfiglio et al., 2012a*); Mozambique, n=16 (JQ684029-JQ684045 were retrieved from the

748   GenBank), South Africa, n=34 (*Horsburgh et al., 2013*) and two additional Nigerian samples

749   mined from GenBank (Accession no. L27731 and L27730). Sizes of the circles are proportional

750   to haplotype frequencies. *m*, refers to number of mutation steps and those not indicated are just

751   one step mutation. Colours indicate the geographical distribution of the sampling locations

752   across Africa, Europe and West Asia as shown by the legend in (b).

753   **Figure 2 The network of 28 cattle samples based on 286 bp of the ZFY Y-chromosome**

754   **region.** Sizes of the circles are proportional to haplotype frequencies and the number of mutation

755   steps in each branch are given. Colours indicate the geographical distribution of the samples as

756   follows: Dark green, Nigeria; Orange, Asia; and Blue, Europe. Source of the data for the Y

757   haplogroups was retrieved from *Nijman et al. 2008*, *Ginja, Telo da Gama & Penedo, (2009)*,

758   MF683853 and MF683854 were retrieved from the GenBank.

759

760   **Table legend**

761   **Table 1** Genetic diversity of cattle in Africa based on mtDNA D-Loop

762   **Table 2** Mutations that describe the distinction of the three major haplogroups of cattle based on

763   ZFY intron 10 gene. The two genomic sites that separate each distinct haplogroup are in bold.

764   The seven mutations observed in Nigerian cattle are italicized.

765

766

Genetic diversity of cattle in Africa based on mtDNA D-Loop

1 **Table 1.** Genetic diversity of cattle in Africa based on mtDNA D-Loop

Population	N	PS	H	HD (SD)	π (SD)	Df
1. Nigeria	119	67	80	0.985(0.005)	0.051(0.029)	3.403
(a) North West[1]	62	44	44	0.984(0.007)	0.076(0.044)	3.338
(b) North East/Central[2]	56	52	43	0.983(0.009)	0.067(0.038)	3.462
(c) West[3]	3*	4	3	1.000(0.272)	0.667(0.598)	2.667
2. Egypt	31	46	30	1.000(0.082)	0.010(0.005)	6.391
3. Mozambique	16	18	15	0.992(0.025)	0.180(0.109)	3.233
4. Ethiopia	126	70	83	0.969(0.009)	0.005(0.003)	3.365
5. South Africa	34	17	24	0.961(0.019)	0.006(0.003)	3.606

4 Note: N = sample size, PS = the number of polymorphic sites, H = the number of haplotypes, HD

5 = haplotype diversity, π = nucleotide diversity, Df = the mean number of nucleotide differences,

6 and SD = standard deviations. [1]Cattle samples from Zamfara, Kano, Katsina, Kaduna and Sokoto

7 States; [2]Samples from Taraba and Jos, Plateau States; [3]Samples from Ibadan, Oyo State. *Two

8 additional downloaded samples from GenBank (Accession no. L27731 and L27730). The

9 estimation of haplotype and nucleotide diversity based on 636 bp mtDNA D-loop sequence was

10 carried out by ARLEQUIN v. 3.5 (Excoffier & Lischer, 2010) software.

Mutations that describe the distinction of the three major haplogroups of cattle based on ZFY intron 10 gene. The two genomic sites that separate each distinct haplogroup are in bold. The seven mutations observed in Nigerian cattle are italicized.

1   **Table 2**. Mutations that describe the distinction of the three major haplogroups of cattle based on

2   ZFY intron 10 gene. The two genomic sites that separate each distinct haplogroup are in bold.

3   The seven mutations observed in Nigerian cattle are italicized.

ZFY intron 10			
Haplogroup	**Reference**	**Alternative**	**Publication**
Y1	C	C	
	GT	--	
Y2	C	**C**	Gotherstrom *et al*. 2005
	GT	**GT**	Ginja *et al*. 2009
Y3	C	**T**	
	GT	GT	
Y3	C	T	
	GT	GT	
g.797	*A*	*G*	
g.786	*A*	*T*	
g.800, 804	*A*	*C*	This study
g.784, 803, 803	*T*	*A*	
g.802, 805	*T*	*G*	
g.802, 803, 804	*T*	*C*	
g.801	*C*	*T*	

Figure 1

Sampling locations of cattle in Nigeria and the network of 420 cattle samples based on 636 bp of the mtDNA D-loop region.

Sampling locations of cattle in Nigeria and the network of 420 cattle samples based on 636 bp of the mtDNA D-loop region. (a) Map of cattle sampling locations in Nigeria (Zamfara, Kano, Katsina, Kaduna, and Sokoto from North West; Taraba and Plateau from North East/Central; and Oyo from the West) and the haplogroup distribution based on mtDNA across Africa . The maps of Africa and Nigeria were generated by an online version of the SmartDraw 2012 software (https://cloud.smartdraw.com/). (b) Median-joining network of 420 cattle samples constructed by using NETWORK v 4.6 (*Bandelt, Forster & Rohl, 1999*) . Reference sequences used for haplotype network analysis included: Europe, n=76 (*Loftus et al., 1994a; Lai et al., 2006; Achilli et al., 2008; Hiendleder, Lewalski & Janke, 2008; Bonfiglio et al., 2012a* and AF034438-AF034446 were retrieved from the GenBank); West Asia, n=16 (*Achilli et al., 2008*); Egypt, n=31 (*Bonfiglio et al., 2012a; Olivieri et al., 2015*); Ethiopia, n=126 (*Dadi et al., 2009; Bonfiglio et al., 2012a*); Mozambique, n=16 (JQ684029-JQ684045 were retrieved from the GenBank), South Africa, n=34 (*Horsburgh et al., 2013*) and two additional Nigerian samples mined from GenBank (Accession no. L27731 and L27730). Sizes of the circles are proportional to haplotype frequencies. *m*, refers to number of mutation steps and those not indicated are just one step mutation. Colours indicate the geographical distribution of the sampling locations across Africa, Europe and West Asia as shown by the legend in (b).
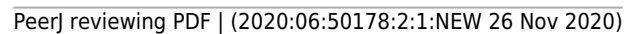
Figure 2

The network of 28 cattle samples based on 286 bp of the ZFY Y-chromosome region

Sizes of the circles are proportional to haplotype frequencies and the number of mutation steps in each branch are given. Colours indicate the geographical distribution of the samples as follows: Dark green, Nigeria; Orange, Asia; and Blue, Europe. Source of the data for the Y haplogroups was retrieved from *Nijman et al. 2008*, *Ginja, Telo da Gama & Penedo, (2009)*, MF683853, MF683854 were retrieved from the GenBank.