Malaysian Journal of Human Genetics (MJHG) | Vol. 4 (1) June 2023

OFFICIAL JOURNAL

ORIGINAL ARTICLE

# Mitochondrial DNA haplogroup determination using Hypervariable Regions I and II of Fulanis and Yorubas in Ilorin, Kwara State and North Central of Nigeria

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**Abstract**

**Introduction:** Mitochondrial DNA (mtDNA) is maternally inherited. It has higher mutation rates than nuclear DNA with its polymorphic sites mainly located in the D-loop control region: non-coding hypervariable regions I (HVI) and II (HVII). HVI and HVII haplogroups are population-specific signatures of the human mitochondrial phylogenetic tree and are relevant to phylogenetic and forensic studies. Nigeria comprises over 250 ethnic groups, out of which the Yorubas and Fulanis are arguably the second and fourth largest ethnic groups respectively. This study examined mtDNA haplotypes and haplogroups in Fulanis and Yorubas resident in Ilorin, Kwara State, North-Central region of Nigeria. **Methods**: Samples were collected with informed consent from non-related 25 Fulani males and 25 Yoruba males whose ethnic groups were confirmed by three generations (paternal and maternal). Non-coding HVI and HVII regions of mtDNA were analysed. **Results**: Two haplogroups (L0a1a2 and L3k1) out of 18 mtDNA haplogroups observed in Fulanis were novel haplogroups not previously observed in Fulanis. Furthermore, L0a1a2, L1b1a, L2c, L2c2, L2c2a1, L2c2b, L3b3, L3e1b1, L3f1b and L3k1 haplogroups were specific to Fulanis. In Yorubas, 14 mtDNA haplogroups including L1b1a10, L1b2a, L1c2b, L1c3c, L2d and L3d1b3a as specific haplogroups were observed. However, L0a1a2, L1b1a, L1b1a18 and L4b1a haplogroups observed previously in Yorubas were not observed in Yorubas of the present study. In addition, L1b, L2a1, L2b, L3b1a, L3b1b, L3d1a1b, L3e2a1b1 and L3e2b haplogroups were observed in both Fulanis and Yorubas. **Conclusion**: Fulanis and Yorubas belong to mtDNA L0 - L3 haplogroups previously established for West Africa.

**Keywords**: Fulanis; Yorubas; mtDNA haplogroups; Hypervariable Region I; Hypervariable Region II.

## Introduction

Mitochondrial DNA (mtDNA) has higher mutation rates than nuclear DNA with its polymorphic sites mainly located in the D-loop control region: non-coding hypervariable regions I (HVI) and II (HVII). HVI and HVII haplogroups are population-specific signatures of the human mitochondrial phylogenetic tree and are of relevance to phylogenetic and forensic studies. mtDNA inheritance is maternal in humans, however, paternal inheritance of mtDNA has been reported in three human families (Johnson *et al.*, 2015). Furthermore, possible paternal mtDNA inheritance across multiple generations has been reported in mice (Rius *et al.*, 2019).

Nigeria is located in West Africa and it is one-sixth of Africa's total population (Alaba *et al.*, 2017). Nigeria is composed of over two hundred and fifty ethnic groups with a population of over one hundred and forty million in 2006 (Alaba *et al.*, 2017; Okolie *et al.*, 2018; Akinlolu *et al.,* 2021). The Hausas, Yorubas, Igbos and Fulanis are the first to fourth largest ethnic groups respectively (Mustapha, 2006; Okolie *et al.*, 2018). The Fulanis are either nomadic herdsmen or settler Fulanis living in permanent settlements (Vicente *et al.*, 2019).

**Received:** 24 May 2023**; Accepted revised manuscript:** 24 July 2023 **Published online:** 14 September 2023
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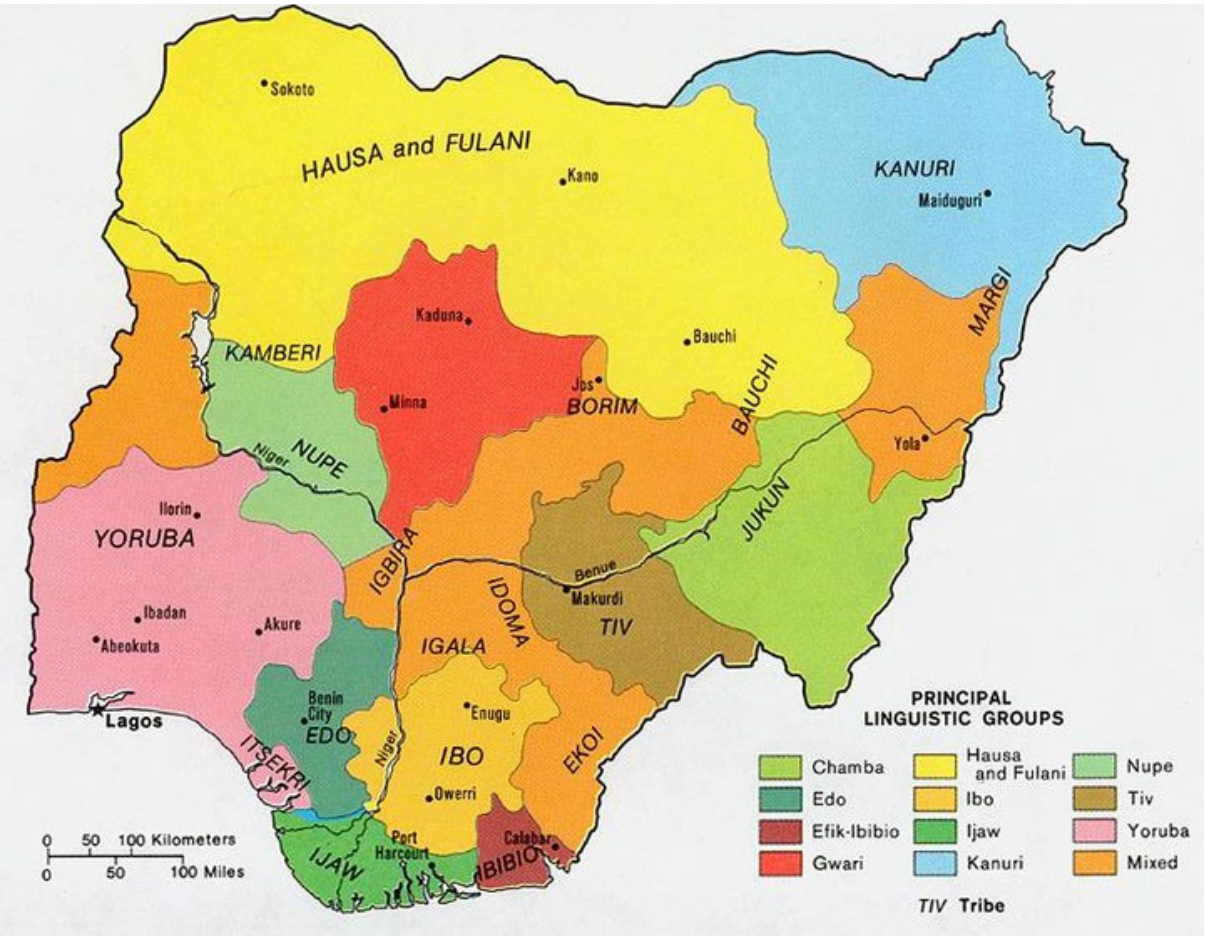


Figure 1. Geographical locations of linguistic groups of Nigeria (Nigeria Linguistic, 1979; Akinlolu *et al.,* 2021).

Environmental factors such as climate conditions have been previously established to have strong effects on genetic structures of ethnic groups (Torres *et al*., 2012; Haber *et al*., 2013). These effects resulted in varied genetic structuring, genetic stratifications and population genetic classifications even within populations historically belonging to the same ethnic and regional groups (Torres *et al*., 2012; Haber *et al*., 2013).

Nigeria is divisible into six distinct North-Central, North-East, North-West South-East, South-South and South-West geo-political regions (Alaba *et al*., 2017; Okolie *et al*., 2018). The climate condition in the core Northern region of Nigeria is arid, while that of Southern Nigeria is tropical rainforest. The climatic differences between Southern and Northern Nigeria may or may not have any epigenetic effect on genetic structures of Nigerian ethnic groups. mtDNA haplotypes or haplogroups specific to ethnic groups are rare, hence regionally confined mtDNA haplotypes or haplogroups have been described.

The Out of Africa hypothesis posits that humans evolved in Africa from where they dispersed to populate the rest of the world (Lahr and Foley, 1994). African populations are therefore expected to have the most diverse genetic polymorphism. The Yorubas and Fulanis are some of the largest ethnic populations in Africa. The Fulanis are nomadic, and they travel across different African regions, and have a long history of intermarriages with their local residents while settled. Fulani and Yorubas settlers though resident in Ilorin, Kwara State in the North Central region of Nigeria may possibly be of ancestral origin outside West Africa and may belong to different mtDNA haplogroups other than reported L0 – L3 haplogroups for West Africa. This follows genetic admixtures via long inter-tribal marriages with another co-resident.

In Nigeria, the Yorubas and Fulanis are arguably the second and fourth largest ethnic groups respectively. No previous study examined mtDNA haplogroups in Fulanis resident in any part of Nigeria. In addition, previous studies examined mtDNA haplogroups in Hausas, Igbos and Yorubas residents in Lagos State, Southwest of Nigeria (Martinez *et al*., 2019), but not in Yorubas who are residents of Ilorin, Kwara State and North-Central of Nigeria. Therefore, this study examined mtDNA haplogroups in Fulanis and Yorubas who are residents of Ilorin, Kwara State and North Central of

 | ISSN (Online): 2716-649X 

Nigeria, to further provide data on the genetic structures of these ethnic groups.

## Materials and Methods

### Ethical approval

This research work was approved by the University Ethical Review Committee (UERC) of the University of Ilorin, Nigeria with approval number; UERC/ASN/2018/1261. Experimental procedures were carried out in accordance with the National Ethics and Operational Guidelines for Research on Human Subjects, the Number code (1947); the World Medical Association Declaration of Helsinki (1964) and its amendments, the Helsinki Declaration of 1975, as revised in 2000 and the Council of International Organization of Medical Sciences (CIOMS) guidelines of 1993 as stated on the research policy of the UERC.

### Determination of sample size and samples collection

Fifty healthy and unrelated subjects (25 and 25 males) were selected from volunteers amongst each of Fulani and Yoruba ethnic groups resident in Ilorin, Kwara State using purposive random sampling technique (Bacchetti *et al*., 2011; Haber *et al*., 2013; Akinlolu, 2016). The purposive technique or judgement sampling method is a non-probability technique which is employed in human invasive studies such as DNA analyses of blood samples which are dependent solely on the number of individuals who chose to volunteer for the study (Bacchetti *et al*., 2011; Haber *et al*., 2013; Akinlolu, 2016). Female subjects were not used in this study because the Fulani customs and traditions will not easily permit access to their women for invasive studies of this nature requiring the taking of blood samples unless for very strong and compelling social and health beneficial reasons amongst others.

The age of Fulanis ranged from 20 - 80 years, while the age of Yorubas ranged from 20 - 25 years. The age difference between Fulani and Yoruba subjects was due to the fact some of the Fulani subjects were non-university students, unlike the Yoruba subjects who were mostly undergraduate students at the University of Ilorin. The aims and objectives of the study were verbally explained to all subjects. Consent forms were distributed to the volunteers seeking their informed consent, and each subject signed the Consent Form to indicate given approval. The genealogies of each volunteer were traced to the third generation based on self-declaration. The inclusion criteria provided strictly that only individuals whose parents, grandparents and great-grandparents are of the Fulani or Yoruba ethnic group were included in this study.

### DNA extraction

Genomic DNA was extracted from collected blood samples according to the manufacturer's instructions using the ZYMO Quick-DNA™ Miniprep Plus Kit (D4068).

### Gel electrophoresis and photography of DNA in agarose gels

Gel electrophoresis was carried out in the laboratory units of the Department of Haematology Obafemi Awolowo University Teaching Hospital, Ile Ife, Osun State, Nigeria. A NanoDrop sphectrophotometer was used for DNA quantification. Agarose gel electrophoresis was used for the separation, identification and purification of 0.5- to 25-kb DNA fragments. DNA was photographed in agarose gels and stained with ethidium bromide by illumination with UV light (>2500 μW/ cm2). The DNA extraction protocol was repeated as appropriate until good qualities of DNA samples were obtained for each sample.

### mtDNA sequencing

mtDNA control regions of HVI and HVII were amplified in a single reaction. The amplified products were incubated and purified with ExoProStar reagent. Complete sequencing of mtDNA HVR1 (16001~16569) and HVR2 (1~574) was carried out using primers designed by Inqaba Biotechnical Industries (Pty) Limited, South Africa (HSMTDNA_15556F: CGAATGATATTTCCTATTCGCCTACA and HSMTDNA_1317R: GGGTACTTGCGCTTACTTTGTAGC). Further purification was achieved using Sephadex columns while fragment separation and detection were via capillary electrophoresis on an ABI3500. All laboratories' procedures and equipment satisfied the conditions of the ISO/IEC 17025 standards.

**Bioinformatics and haplogroups analyses**

mtDNA haplogroups analysis was conducted using mthap version 0.19b (2015-05-11) as previously described by Oven and Kayser, 2008. Observed Haplogroups were assigned on EMPOP database v4/R12 and in comparison with standard references on PhyloTree Build 17 (2016-02-18) +mods.

## Results

### mtDNA haplogroups in Fulani subjects

Eighteen (18) mtDNA haplogroups were observed in 25 Fulani subjects resident in Ilorin, Kwara State of North-Central region of Nigeria as presented in Tables 1 - 4. L1b and L3e2b were the most common haplogroups with a percentage frequency of 12 and were each observed in 3 out of 25 Fulanis (Table 5). Less common were L3b1a, L3b1b and L3e2a1b1 haplogroups with a percentage frequency of 8 which were each observed in 2 out of 25 Fulanis (Table 5). In addition, L0a1a2, L1b1a, L2a1, L2b, L2c, L2c2, L2c2a1, L2c2b, L3b3, L3d1a1b, L3e1b1, L3f1b and L3k1 haplogroups were each observed in 1 out of 25 Fulanis with the percentage frequency of 4 (Table 5).

### mtDNA haplogroups in Yoruba subjects

Fourteen (14) mtDNA haplogroups were observed in 25 Yoruba subjects resident in Ilorin, Kwara State of North-Central region of Nigeria as presented in Tables 1 - 4. L1b was the most common haplogroup with a percentage frequency of 32 and was observed in 8 out of 25 Yorubas (Table 5). Furthermore, L2b, L3b1a, L3e2a1b1 and L3e2b haplogroups were each observed in 2 out of 25 Yorubas with a percentage frequency of 8 (Table 5). In addition, L1b1a10, L1b2a, L1c2b, L1c3c, L2a1, L2d, L3b1b, L3d1a1b and L3d1b3a haplogroups were each observed in 1 out of 25 Yorubas with the percentage frequency of 4 (Table 5).

### mtDNA haplogroups common to Fulani and Yoruba subjects

L1b, L2a1, L2b, L3b1a, L3b1b, L3d1a1b, L3e2a1b1 and L3e2b haplogroups were observed in both Fulani and Yoruba subjects as presented in Tables 1 – 5. The defining markers and variants from which the mtDNA haplogroups in Fulani and Yoruba subjects were derived, and detailed in the Supplementary Data (Mitochondrial Variants in Fulani and Yoruba Subjects). No unique variants or heteroplasmy were detected in this study.

 | ISSN (Online): 2716-649X 

Table 1. mtDNA haplogroups in Fulani and Yoruba subjects of the present study in comparison with Fulanis, Yorubas, West Africans and African-Americans reported in previous studies.

| **Haplogroups** | **Fulanis (present study)** | **Yorubas (present study)** | **Fulanis (Cerny *et al.*, 2006)** | **Yorubas (Martinez *et al.*, 2019)** | **West African Nations (Rosa *et al.*, 2004)** | **African-Americans (Johnson *et al.*, 2015)** |
|---|---|---|---|---|---|---|
| L0 | Absent | Absent | Absent | Absent | Absent | Present |
| Loa | Absent | Absent | Absent | Absent | Present | Absent |
| L0a1a2 | Present | Absent | Absent | Present | Absent | Absent |
| L1 | Absent | Absent | Present | Absent | Present | Present |
| L1b | Present | Present | Present | Absent | Present | Present |
| L1b1a | Present | Absent | Absent | Absent | Absent | Absent |
| L1b1a10 | Absent | Present | Absent | Absent | Absent | Absent |
| L1b1a18 | Absent | Absent | Absent | Present | Absent | Absent |
| L1b1a+189 | Absent | Absent | Absent | Present | Absent | Absent |
| L1b2a | Absent | Present | Absent | Present | Absent | Absent |
| L1c | Absent | Absent | Present | Absent | Present | Present |
| L1c1 | Absent | Absent | Absent | Present | Absent | Absent |
| L1c2b | Absent | Present | Absent | Absent | Absent | Absent |
| L1c3a | Absent | Absent | Absent | Present | Absent | Absent |
| L1c3b | Absent | Absent | Absent | Present | Absent | Absent |
| L1c3c | Absent | Present | Absent | Absent | Absent | Absent |
| L2 | Absent | Absent | Absent | Absent | Absent | Present |
| L2a | Absent | Absent | Present | Absent | Absent | Present |
| L2a1 | Present | Present | Absent | Present | Absent | Absent |
| L2a1+143+@16309 | Absent | Absent | Absent | Present | Absent | Absent |
| L2a1+16189+(16192) | Absent | Absent | Absent | Present | Absent | Absent |
| L2a1a3c | Absent | Absent | Absent | Present | Absent | Absent |

 | ISSN (Online): 2716-649X 

Table 2. mtDNA haplogroups in Fulani and Yoruba subjects of the present study in comparison with Fulanis, Yorubas, West Africans and African-Americans reported in previous studies (continued).

| **Haplogroups** | **Fulanis (present study)** | **Yorubas (present study)** | **Fulanis (Cerny *et al.*, 2006)** | **Yorubas (Martinez *et al.*, 2019)** | **West African Nations (Rosa *et al.*, 2004)** | **African-Americans (Johnson *et al.*, 2015)** |
|---|---|---|---|---|---|---|
| L2a1b1a | Absent | Absent | Absent | Present | Absent | Absent |
| L2a1c1 | Absent | Absent | Absent | Present | Absent | Absent |
| L2b | Present | Present | Present | Absent | Present | Present |
| L2b1a | Absent | Absent | Absent | Present | Absent | Absent |
| L2c | Present | Absent | Present | Absent | Present | Present |
| L2c2 | Present | Absent | Absent | Present | Absent | Absent |
| L2c2a1 | Present | Absent | Absent | Absent | Absent | Absent |
| L2c2b | Present | Absent | Absent | Absent | Absent | Absent |
| L2d | Absent | Present | Present | Absent | Absent | Absent |
| L2d+16129 | Absent | Absent | Absent | Present | Absent | Absent |
| L2e | Absent | Absent | Absent | Present | Absent | Present |
| L3 | Absent | Absent | Present | Absent | Present | Present |
| L3b | Absent | Absent | Absent | Present | Present | Present |
| L3b/d | Absent | Absent | Present | Absent | Absent | Absent |
| L3b1a | Present | Present | Absent | Present | Absent | Absent |
| L3b1b | Present | Present | Absent | Absent | Absent | Absent |
| L3b1a8 | Absent | Absent | Absent | Present | Absent | Absent |
| L3b2 | Absent | Absent | Absent | Present | Absent | Absent |
| L3b2a | Absent | Absent | Absent | Present | Absent | Absent |
| L3b3 | Present | Absent | Absent | Absent | Absent | Absent |
| L3c | Absent | Absent | Absent | Absent | Absent | Present |
| L3d | Absent | Absent | Absent | Present | Present | Present |

 

Table 3. mtDNA haplogroups in Fulani and Yoruba subjects of the present study in comparison with Fulanis, Yorubas, West Africans and African-Americans reported in previous studies (continued).

| **Haplogroups** | **Fulanis (present study)** | **Yorubas (present study)** | **Fulanis (Cerny *et al.*, 2006)** | **Yorubas (Martinez *et al.*, 2019)** | **West African Nations (Rosa *et al.*, 2004)** | **African-Americans (Johnson *et al.*, 2015)** |
|---|---|---|---|---|---|---|
| L3d1a1b | Present | Present | Absent | Absent | Absent | Absent |
| L3d1b3a | Absent | Present | Absent | Absent | Absent | Absent |
| L3d1d | Absent | Absent | Absent | Present | Absent | Absent |
| L3d2 | Absent | Absent | Absent | Present | Absent | Absent |
| L3d5 | Absent | Absent | Absent | Present | Absent | Absent |
| L3e | Absent | Absent | Present | Absent | Present | Present |
| L3e1a | Absent | Absent | Absent | Present | Absent | Absent |
| L3e1a3b | Absent | Absent | Absent | Present | Absent | Absent |
| L3e1b1 | Present | Absent | Absent | Absent | Absent | Absent |
| L3e2 | Absent | Absent | Absent | Present | Absent | Absent |
| L3e2a1 | Absent | Absent | Absent | Present | Absent | Absent |
| L3e2a1b1 | Present | Present | Absent | Absent | Absent | Absent |
| L3e2b | Present | Present | Absent | Present | Absent | Absent |
| L3e2a1b1 | Absent | Absent | Absent | Absent | Present | Absent |
| L3e3 | Absent | Absent | Absent | Present | Absent | Absent |
| L3e4a | Absent | Absent | Absent | Present | Absent | Absent |

Table 4. mtDNA haplogroups in Fulani and Yoruba subjects of the present study in comparison with Fulanis, Yorubas, West Africans and African-Americans reported in previous studies (continued).

| **Haplogroups** | **Fulanis (present study)** | **Yorubas (present study)** | **Fulanis (Cerny *et al.*, 2006)** | **Yorubas (Martinez *et al.*, 2019)** | **West African Nations (Rosa *et al.*, 2004)** | **African-Americans (Johnson *et al.*, 2015)** |
|---|---|---|---|---|---|---|
| L3f1b | Present | Absent | Absent | Absent | Absent | Absent |
| L3f1b+16292 | Absent | Absent | Absent | Present | Absent | Absent |
| L3f1b+16292+150 | Absent | Absent | Absent | Present | Absent | Absent |
| L3f1b1 | Absent | Absent | Absent | Present | Absent | Absent |
| L3f1b1a | Absent | Absent | Absent | Present | Absent | Absent |
| L3h | Absent | Absent | Absent | Absent | Present | Present |
| L3k1 | Present | Absent | Absent | Absent | Absent | Absent |
| L3x | Absent | Absent | Absent | Absent | Absent | Present |
| L4 | Absent | Absent | Absent | Absent | Absent | Present |
| L4b1a | Absent | Absent | Absent | Present | Absent | Absent |

 | ISSN (Online): 2716-649X 

Table 5. Percentage of mtDNA haplogroups in Fulani and Yoruba subjects.

| Haplogroups | Percentage Frequency in Fulanis | Percentage Frequency in Yorubas |
|---|---|---|
| L0a1a2 | 4 | 0 |
| L1b | 12 | 32 |
| L1b1a | 4 | 0 |
| L1b1a10 | 0 | 4 |
| L1b2a | 0 | 4 |
| L1c2b | 0 | 4 |
| L1c3c | 0 | 4 |
| L2a1 | 4 | 4 |
| L2b | 4 | 8 |
| L2c | 4 | 0 |
| L2c2 | 4 | 0 |
| L2c2a1 | 4 | 0 |
| L2c2b | 4 | 0 |
| L2d | 0 | 4 |
| L3b1a | 8 | 8 |
| L3b1b | 8 | 4 |
| L3b3 | 4 | 0 |
| L3d1a1b | 4 | 4 |
| L3d1b3a | 0 | 4 |
| L3e1b1 | 4 | 0 |
| L3e2a1b1 | 8 | 8 |
| L3e2b | 12 | 8 |
| L3f1b | 4 | 0 |
| L3k1 | 4 | 0 |

## Discussion

Previous studies on mtDNA population genetics emphasized the tested Fulanis of Chad, Cameroon, and Burkina Faso (West African countries) (Cerny *et al.*, 2006), and Yorubas of Ibadan and Lagos (South-West, Nigeria) (Martinez *et al.*, 2019) as residents of their respective study locations. Hence, the mtDNA haplogroups of Fulanis and Yorubas who are residents of Ilorin, Kwara State, and North-Central of Nigeria presented in this study are novel.

L0a1a2, L1b1a, L2c, L2c2, L2c2a1, L2c2b, L3b3, L3e1b1, L3f1b and L3k1 haplogroups were observed only in Fulanis (Tables 1 - 4). Previous studies showed that Fulanis of Chad, Cameroon, and Burkina Faso, belonged to mtDNA haplogroups L1, L1b, L1c, L2a, L2b, L2c, L2d, L3, L3b/d, L3e and L3f (Cerny *et al.*, 2006). Hence, this study observed new major mtDNA haplogroups in Fulanis such as L0a1a2 and L3k1 which were not observed in previous studies on Fulanis.

 | ISSN (Online): 2716-649X 

L1b1a10, L1b2a, L1c2b, L1c3c, L2d and L3d1b3a haplogroups were observed only in Yorubas (Tables 1 - 4). Comparatively, the L1 - L3 major and sub-lineage mtDNA haplogroups observed in Yorubas of this study were similar to those previously observed in Yorubas residents in Lagos, Lagos State of Southwest of Nigeria by Martinez *et al.* 2019. However, mtDNA haplogroups sub-lineages observed previously in Yorubas such as L0a1a2, L1b1a, L1b1a18 and L4b1a (Martinez *et al.*, 2019), were not observed in Yorubas evaluated in this study (Tables 1 - 4).

L1b, L2a1, L2b, L3b1a, L3b1b, L3d1a1b, L3e2a1b1 and L3e2b haplogroups were commonly observed in both Fulanis and Yorubas (Tables 1 - 5). The L0 - L3 major and sub-lineage mtDNA haplogroups observed in Fulanis and Yorubas evaluated in this study were similar to those previously observed in indigenous peoples of West African and other African nations (Ethiopia, Guinea, Kenya, Mozambique and Senegal) (Rosa *et al.*, 2004), Khoisans of South-Africa (Chen *et al.*, 2000), and African-Americans (Johnson *et al.*, 2015) (Tables 1 - 4). Hence, the observations of this study further confirmed that Fulanis and Yorubas of Nigeria belong to the L0 – L3 mtDNA haplogroups indicating that these haplogroups are population-specific signatures of Fulanis and Yorubas on the human mitochondrial phylogenetic tree which are of relevance to phylogenetic and forensic studies.

## Conclusion

This study provides data on mtDNA haplogroups for the Fulanis and Yorubas who are residents of Ilorin, Kwara State, and North-Central of Nigeria, and further confirms that Fulanis and Yorubas belong to mtDNA L0 - L3 haplogroups previously established for West Africa.

## Limitations of the study and future research direction

This study is limited to genotyping data of mtDNA haplogroups in Fulanis and Yorubas. Mitochondrial DNA haplogroup determination using Hypervariable Region III and genome-wide association studies shall be carried out in future research studies to provide further data on the genetic structures of Fulanis and Yorubas of Nigeria.

## Acknowledgements

The author thanks all Fulani and Yoruba subjects who donated blood samples. The author acknowledges the technical support of Inqaba Biotechnical Industries (Pty) Limited, Pretoria, South Africa which provided the primers for mtDNA HVI and HVII, and also carried out the sequencing procedures. In addition, the author strongly acknowledges the support of him undergraduate project students at the study institutions who assisted with samples collection for this study.

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