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Short communication

Molecular genetic diversity analysis of the Nigerian laughing dove (*Streptopelia senegalensis)* and related species using selected mitochondrial genes

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A R T I C L E I N F O

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A B S T R A C T

Nigerian laughing doves (*Streptopelia senegalensis*) are small birds with long tail and living in bushes of the Sub-Saharan regions of African continent, the middle East and Asia, especially India. They are used for food, medicinal and religious purposes in Nigeria. Despite their usage, there is a lack of information on the genetic diversity of laughing doves in Nigeria. This study investigates taxonomic order and diversity of Nigerian laughing doves based on the mitochondrial cytochrome oxidase subunit I (*COI*) and cytochrome B (*CYTB*). The results showed 20 haplotypes within the 28 Nigerian coupled with Global Streptopelia genus using concatenated sequences. The Nigerian laughing dove constitute 16 distinct haplotypes. The haplotype diversity was 0.743 ± 0.070, and nucleotide diversity 0.154 ± 0.101 within Nigerian population using *COI* sequences. Phylogenetic tree showed that Nigerian laughing doves were in the same monophyletic clade with other *Streptopela orientalis, S. decocto* and *S. chinensis*; and this confirmed that Nigerian laughing doves might have shared descendant. The median-joining network further grouped Nigerian laughing doves into two: the first group consisting of Nigerian populations only, while the second group are with Saudi Arabian and Djiboutian populations. Population expansion was revealed in Nigerian dove individuals. This study revealed 16 unique haplotypes among Nigerian laughing dove population using concatenated sequences. Interestingly, *CYTB* showed clustering in African laughing doves (For instance, Nigerian individuals shared haplotypes with Sao Tome and Principe, an island country in the Gulf of Guinea, the western equatorial coast of Central Africa). The current data is the first report on genetic diversity of Nigerian laughing dove using mitochondrial *COI* and *CYTB* genes.

1. Introduction

The laughing dove (*Streptopelia senegalensis*) is a reddish-brown bird with blue markings on its wings, a white edge on its long tail, purplish legs, and a black beak. It is a widespread species in Sub-Sahran Africa, the Middle East, and southern Asia (Zannetos et al., 2023). However, some species have been introduced to the New World and Australia (Johnson et al., 2001). This small long-tailed dove is also found in dry scrub and semi-desert habitats where pairs can often be seen feeding on the ground (Ali and Ripley, 1995). The phenotypic features of white doves are similar with the wild-type brown laughing doves except from coloration (Aktay et al., 2017).

Abbreviations: COI, Cytochrome C Oxidase I; *CYTB*, Cytochrome B; PCR, Polymerase Chain Reaction; FTA, Flinders Technology Associates; DNA, Deoxyribonucleic acid; nHT, Number of haplotypes; HTdiv, Haplotype diversity; ndiv, Nucleotide diversity; ML, Maximum Likelihood; F_{ST}, Fixation index; MJ, Median-joining.

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Table 1
Genetic diversity of Nigerian and global *Streptopelia* genus populations based on 618 bp of *COI* gene.

Population	Size[a]	nHT[b]	HTdiv (SD)[c]	πdiv (SD)[d]	*D*[e]	*Fs*[f]	MNPD (SD)[g]
Nigeria	28	8	0.743(0.070)	0.154(0.101)	−1.326*	−1.697	1.849(1.093)
Saudi Arabia	3	3	1.000(0.272)	0.666(0.598)	0.000	−0.341	2.667(1.919)
Afghanistan	5	3	0.700(0.218)	0.400(0.398)	−0.973*	−0.829	0.800(0.681)
Djibouti	2	2	1.000(0.500)	1.000(1.225)	0.000	0.693	2.000(1.732)
Pakistan	1	1	–	–	–	–	–
India	1	1	–	–	–	–	–
Group A[h]	19	4	0.521(0.123)	0.049(0.046)	−0.867	−1.264	0.585(0.492)
Group B[i]	9	4	0.583(0.183)	0.162(0.115)	−1.541*	0.331	1.944(1.214)

[a] Total number of samples in each region.
[b] Number of haplotypes.
[c] Haplotype diversity (standard deviation).
[d] Nucleotide diversity (standard deviation).
[e] Tajima's *D* test of selective neutrality.
[f] Fu's *Fs* test of selective neutrality.
[g] Mean number of pairwise differences (standard deviation).
[h] Group A - Nigerian sub-population based on the phylogenetic tree.
[i] Group B - Nigerian sub-population based on the phylogenetic tree. * indicates statistical significance at 5 % level.

European turtle dove are subject to experience demographic fluctuations (trait that is keen to anthropogenic influences) (Calderón et al., 2016). In a complete metagenome of Oriental turtle dove (*Steptopelia orientalis*) using two barcoding genes (*Cox1* and *Cytb*), the authors reported that the genetic distances coexist with morphological, behavioral and phylogenetic analyses suggesting the function of chinensis and senegalensis to the Spilopelia (Huang et al., 2016). In addition, manipulating the ecological niches such as nests placement, nesting areas and nests orientations revealed convergence and competition among three (collared, turtle and laughing) doves in Ouargla region (Northeast of Algerian Sahara) (Ahlame et al., 2020) and some behaviors showed phylogenetic relationships despite diversity and rapid fast evolution of vocalizations in *Streptopelia spp* (Johnson et al., 2001).

Several techniques have been developed to estimate the genetic variation or polymorphism in population and hence the genetic relationship among the population; these method include biochemical polymorphism (Adeleke et al., 2011; Ige et al., 2013), and DNA hybridization (Farrag et al., 2010). The changes in hematology, plasma biochemistry, and erythrocyte osmotic fragility of the Nigerian laughing dove (*Streptopelia senegalensis*) have examined (Azeez et al., 2013). Studies have utilized the Cytochrome *c* oxidase I (*COI*) gene for the identification and discovery of species (Pentinsaari et al., 2016; Ptaszyńska et al., 2012) and to examine the genetic diversity of Eurasian (Nagai et al., 2020) and Pakistani (Awan et al., 2013) collared doves. Moreover, Cytochrome B (*CYTB*) was utilized for identification and differentiation of poultry species including laughing dove (Awad et al., 2015) and analyses of complete mitochondrial genome Oriental turtle dove *Strptopelia orientalis* (Huang et al., 2016).

However, there is paucity of information on the genetic diversity of laughing dove population in Nigeria. Hence, the current study investigates genetic diversity of Nigerian laughing doves using concatenated sequences of cytochrome *b* (*CYTB*) and cytochrome oxidase subunit 1 (*COI*) (DNA barcoding).

2. Materials and methods

28 laughing doves (*Streptopelia senegalensis*) were caught using a locally constructed birds catcher at Fair and Firm Farm Tanke, Oke-Odo Ilorin Kwara State, Nigeria. Blood samples were carefully collected through wing venipuncture of each bird on FTA cards (Supplementary Fig. 1). Genomic DNA was extracted from whole blood preserved on the FTA cards. DNA quantification, PCR amplification, and Sanger sequencing were done (Appendix S1). The *COI* and *CYTB* sequences were concatenated using MEGA version 6.06 (Tamura et al., 2013). All the sequences were compared, and haplotypes identified using DnaSP 5.10.1. Further, we calculated genetic diversity across all studied populations. Phylogenetic tree was constructed to determine the relationship of 28 Nigerian laughing doves with other dove samples retrieved from public database (Table S1). We visualized the relationships between haplotypes of the 37 laughing doves concatenated sequences by constructing median-joining network (Bandelt et al., 1999) implemented in Network 5.0.1.1 (www.fluxus-engineering.com). The population dynamics and demographic patterns, demographic statistical parameters for Tajima's *D*, Fu's *Fs* tests, and population F_{ST} were calculated using *COI* and *CYTB* sequences separately (Appendix S1).

Please check Appendix S1 for detailed materials and methods.

3. Results and discussion

The 37 *Streptopelia senegalensis* concatenated sequences from Nigeria and other retrieved sequences from Saudi Arabia, Afghanistan, India, Djibouti, Pakistan, and *S. chinensis* (as outgroups) classify into 20 haplotypes (Table S2). The 26 Nigerian laughing doves [*de novo* (GenBank: OK255714 - OK255740)] constitute 16 distinct haplotypes (HT1-HT16) (Table S2). HT17 appears in *Streptopelia chinensis* only while largest frequency (HT2) occurs only in Nigerian population (Table S2). This might happen due to concurrent gene flow. Similar patterns were observed in the genetic structure of Eurasian Collared Dove (*S. decaocto*) (Bagi et al., 2018). Moreso, previous study suggested that *Streptopelia chinensis* and *Streptopelia senegalensis* should be apportioned to genus Spilopelia based on the recent taxonomic system of IUCN (IUCN, 2023).

Further, we detected 19 haplotypes including eight districts (HT2-HT9) in Nigerian population based 618 bp of *COI* sequences (Table S3). Subsequently, 36 haplotypes were revealed in 68 Nigerian and Global *Streptopelia senegalensis* using 286 bp of *CYTB* gene (Table S4). The largest haplotype (HT1) was shared between Nigerian and other *Streptopela senagalensis.*

Using *COI* sequences, the estimated haplotype diversity (HTdiv) in Nigerian individuals is 0.743 ± 0.070 which is almost like Afghanistan (0.700 ± 0.218) but lower than Saudi Arabian (1.000 ± 0.272) and Djiboutian populations (1.000 ± 0.500) (Table 1). While the Nigerian nucleotide diversity value 0.154 ± 0.101 is the lowest.

The maximum likelihood (ML) tree of 37 Nigerian and global Streptopelia genus based on concatenated sequences shows a monophyletic clade relationship within the Nigerian laughing dove and a polyphyletic clade relationship between *Streptopela orientalis, decocto* and *chinensis* (Fig. 1A). The results shows that most of the Nigerian laughing dove individuals are closely related. The median-joining network of two concatenated mitochondrial regions of 37 *S. senegalensis* together with *S. chinensis, S. decaocto,* and *S. orientalis* revealed 20 different haplotypes, most of which were singleton (Fig. 1B). H2 (1403-S + S + NIG) showed the highest frequency which

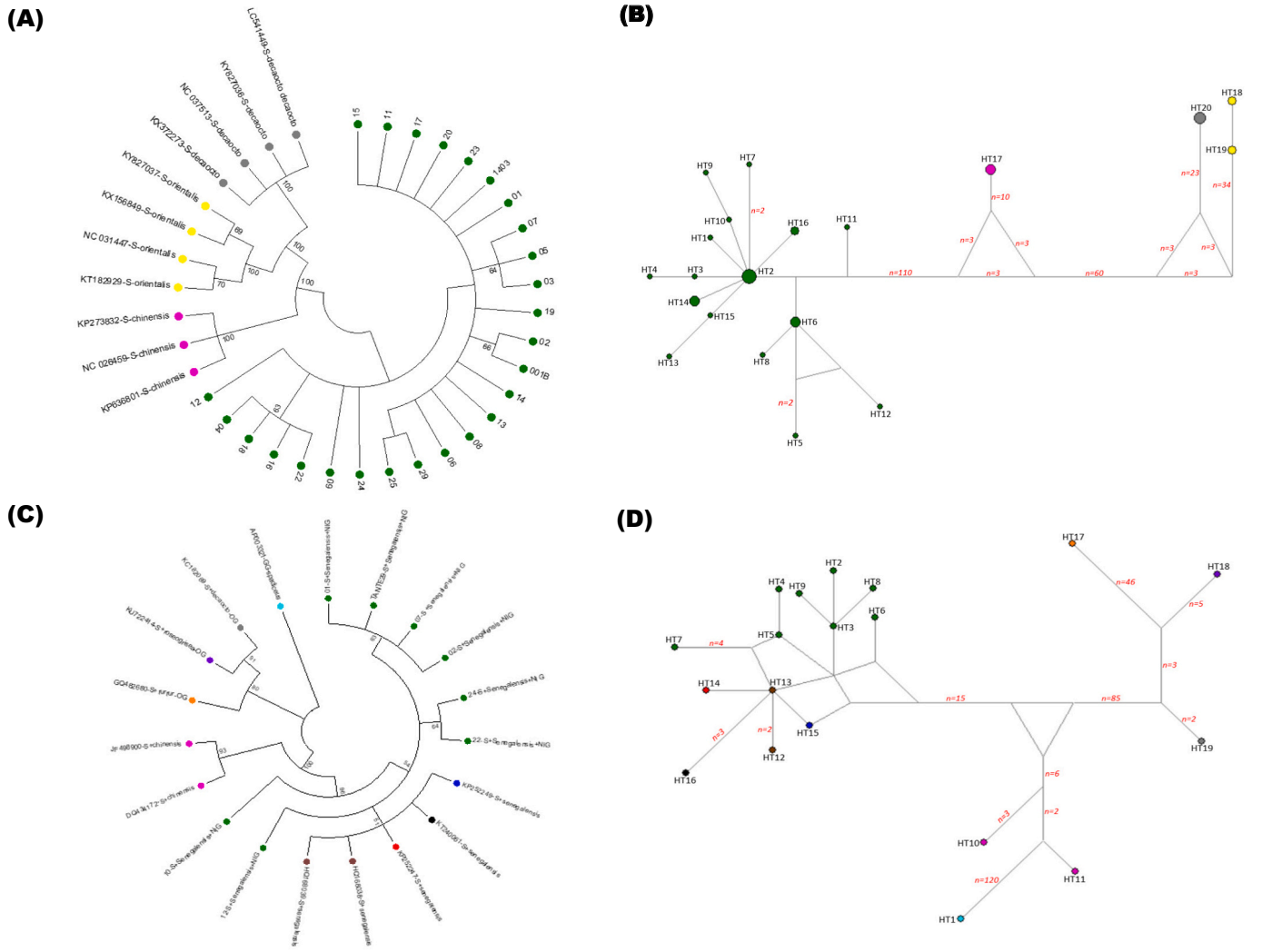


Fig. 1. (A) Maximum Likelihood (ML) for 37 Laughing doves (*Streptopelia senegalensis*) from Nigeria and globally based on two concatenated mitochondrial regions. Colours indicate location: Green *Streptopelia senegalensis* from Nigeria; Pink is *S. chinensis*; Grey is *S. decaocto*; Yellow is *S. orentalis*. (B) Median-joining network for 37 Laughing doves (*Streptopelia senegalensis*) from Nigeria and globally based on two concatenated mitochondrial regions using NETWORK version 4.6. 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 location: Green *Streptopelia senegalensis* from Nigeria; Pink is *S. chinensis*; Grey is *S. decaocto*; Yellow is *S. orentalis*. (C) Maximum Likelihood (ML) for 19 haplotypes sharing of 51 Nigerian and global Streptopelia genus based on cytochrome oxidase subunit 1 (*COI*). Colours indicate location: Green is *Streptopelia senegalensis* from Nigeria; brown is *S. senegalensis* from Saudi Arabia; red is *S. senegalensis* from Afghanistan; black is *S. senegalensis* from India; blue is *S. senegalensis* from Djibouti; pink is (*S. chinensis*); Orange is *S. turtur*; Purple is *S. roseogrisea*; Grey is *S. decaocto*; Light blue is Out group (*Gallus gallus spadiceus*). (D) Median-joining network for 19 haplotypes sharing of 51 Nigerian and global Streptopelia genus based on cytochrome oxidase subunit 1 (*COI*) using NETWORK version 4.6. 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 location: Green is *Streptopelia senegalensis* from Nigeria; brown is *S. senegalensis* from Saudi Arabia; red is *S. senegalensis* from Afghanistan; black is *S. senegalensis* from India; blue is *S. senegalensis* from Djibouti; pink is (*S. chinensis*); Orange is *S. turtur*; Purple is *S. roseogrisea*; Grey is *S. decaocto*; Light blue is Out group (*Gallus gallus spadiceus*). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was present only in Nigerian laughing dove population. The next prominent haplotype, HT6 (22-S + S + NIG) and HT14 (07-S + S + NIG) were present in six Nigerian laughing dove (Fig. 1B).

A phylogenetic tree based on 19 haplotypes of laughing dove from Nigeria and global was constructed based on *COI* sequences was done. A few minor clustering of six individuals from Nigeria with other *S. senegalensis* from India, Djibouti, Saudi Arabia, and Afghanistan was observed in the same clade (Fig. 1C). We constructed median-joining network of *COI* for19 laughing dove from Nigeria and other countries using *Gallus gallus spadiceus* as outgroup. We observed singleton haplotype (Fig. 1D).

Further, we constructed ML tree for 68 laughing dove from Nigerian and global using *CYTB*. We observed that laughing dove individuals from Nigeria clustered with those from Sao Tome and Principe in Africa (Fig. S3). Recent study revealed that there is a phylogenetic tree relationships between West African mud turtle on the island of Sao Tome and Principe (Guedes et al., 2023). Additionally, median-joining network of 68 laughing dove from Nigeria and global was constructed using their *CYTB* sequences. Based on this, HT1-HT10 exhibits star-like patterns indicating rapid population expansion (Fig. S3). In earlier study, genomic signatures of unstable demographic history combined with a current effective population size contraction in turtle dove which support evidence for its sensitive conservation situation (Calderón et al., 2016). Additionally, other poultry species in Nigeria like Muscovy duck has shown a similar pattern (Adeola et al., 2022). There is evidence of gene flow between African and Asian surrounding areas including Saudi Arabia (Abu-Amero et al., 2009).

Based on *COI* sequences, the Tajima's *D* and Fu's Fs in Nigerian

laughing doves are −1.326 ($P < 0.05$) and −1.697 ($P > 0.05$), respectively (Table 1). The value signal demographic expansion in Nigerian laughing dove. Further analysis within Nigerian population revealed population expansion in group B population Tajima's *D* (Tajima, 1989) -1.541 ($P < 0.05$) (Table 1). Recent study has also revealed population expansion in Nigerian wild grasscutter populations (Adedeji et al., 2023).

We detected high haplotype (0.743 ± 0.070) and low nucleotide diversity (0.154 ± 0.101) values in the Nigerian population using *COI* sequences suggesting fast expansion from an ancestral population (Arbogast, 2001) which might happen due to concurrent gene flow. In a previous study on European *S. turtur*, a similar thread was observed in their genetic diversity using mitochondrial DNA cytochrome-b and D-loop (Prakas et al., 2021). Perhaps, the Nigerian laughing dove population might be experiencing expansion (Frankham, 2005).

Further studies could utilize other genetic markers with more individuals to confirm the genetic diversity and comprehensive demographic history of the Nigerian laughing dove populations.

CRediT authorship contribution statement

Foluke E. Sola-Ojo: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Ibraheem A. Abubakar:** Methodology, Writing – original draft. **Semiu F. Bello:** Conceptualization, Formal analysis, Investigation, Validation, Visualization, Writing – review & editing. **Uthman Oladipo:** Methodology. **Sule Bisola:** Methodology, Writing – original draft. **Isiaka H. Fatimoh:** Methodology, Writing – original draft. **Wasiu A. Olaniyi:** Formal analysis, Investigation, Writing – review & editing. **Adesina M. Oluwasegun:** Formal analysis, Investigation, Writing – review & editing. **Ming-Shan Wang:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Adeniyi C. Adeola:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of competing interest

The authors have no conflicts of interest.

Data availability

Data will be made available on request.

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Compliance with ethical standards

All experimental procedures were performed in accordance with the University Ethical Review Committee, University of Ilorin, Nigeria (UERC/ASN/2021/2161) and Research Guidelines for the Institutional Review Board of Kunming Institute of Zoology, Chinese Academy of Sciences (SMKY-20160105-11).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.genrep.2024.102003.

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