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Genetic diversity and population structure of the native Western African honeybee (*Apis mellifera adansonii* Latreille, 1804) in Nigeria based on mitochondrial COI sequences

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ABSTRACT

The west African honeybee, *Apis mellifera adansonii*, is a subspecies of western honeybees that live in a wide range of habitats. In Nigeria, there is a lack of knowledge of its population genetics. Thus, we examined its mitochondrial genetic diversity and population structure using the subunit I of the *cytochrome c oxidase* gene. Our results showed an overall high level of genetic diversity (haplotype diversity = 0.702) of *A. m. adansonii* in Nigeria. Analyses revealed a low level of population genetic structuring ($\Phi ST = 0.31986$) across the geographical regions of Nigeria. Results showed that a substantial degree of haplotype diversity was evident in the populations from the Middle belt of Nigeria. However, individuals from southern Nigeria expressed low haplotype diversity. The mismatch distribution and historical demographics supported evidence of population expansion of *A. m. adansonii* in Nigeria. The matrilineal genealogy demonstrated a lack of clear patterns of population structuring. In sum, our analysis showed a high degree of genetic diversity with no population divergence within *A. m. adansonii* from Nigeria. Further study involving additional mitochondrial and nuclear gene markers could aid to elucidate gene flow patterns among populations of *A. m. adansonii* across its range in West Africa.

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1. Introduction

The western honeybee, *Apis mellifera* Linnaeus, 1758 (Hymenoptera, Apidae), is the most common species of honeybees in the world (Engel 1999; Lo et al., 2010). Like all honeybees, *A. mellifera* is a eusocial insect that is economically and ecologically relevant in the ecosystem (Rand et al., 2015; Haddad et al., 2016; Cridland et al., 2017; Eimanifar et al., 2018). Believed to have originated in Africa (Whitfield et al., 2006) or Asia (Han et al., 2012), *A. mellifera* is now found on all continents due to human assistance, except for Antarctica (Mortensen et al., 2013). Studies have shown reports of about 31 subspecies of *A. mellifera* occurring worldwide (Engel 1999; Nielsen et al., 2000; Abramson & Aquino 2002; Sheppard & Meixner 2003; Ivanova et al., 2010; Meixner et al., 2011; Chen et al., 2016; Fontana et al., 2018). Based on extensive sampling and detailed morphometric and genetic analyses, these subspecies have been divided into at least seven distinct groups: (A) African group, (C) north Mediterranean group, (M) northern and western European group, (O and Z) Middle Eastern group, (Y) in Ethiopia, an additional group in Syria and a new African subgroup described in the South-West Indian Ocean region (Ruttner et al., 1978; Ruttner

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1988; Garnery et al., 1992; Franck et al., 2000; Franck et al., 2001; Alburaki et al., 2011; 2013; Techer et al., 2017). This outstanding genetic diversity could relate to the species' adaptation to the local environmental conditions (Whitfield et al., 2006; Gupta et al., 2014).

Earlier mitochondrial DNA studies confirmed the African mitochondrial lineage A as the most genetically diverse among the evolutionary groups of *A. mellifera* (Ruttner et al., 1978; Ruttner 1988; Garnery et al., 1993; Smith et al., 1991; Arias & Sheppard 1996; De la Rua et al., 1998). According to Engel (1999), Meixner et al. (2011), and Fontana et al. (2018), 11 valid subspecies are now grouped under the African mitochondrial lineage as follows: *A. m. adansonii* Latreille, 1804 in western Africa, *A. m. capensis* Eschscholtz, 1822 in southern Africa, *A. m. intermissa* Buttel-Reepen, 1906 and *A. m. sahariensis* Baldensperger, 1932 in northern Africa, *A. m. jemenitica* Ruttner, 1976, and *A. m. lamarckii* Cockerell, 1906 in north-eastern Africa, *A. m. monticola* Smith, 1961 and *A. m. litorea* Smith, 1961 in south-eastern Africa, *A. m. scutellata* Lepeletier, 1836 in southern and eastern Africa, *A. m. simensis* Meixner et al., 2011 in Ethiopia and *A. m. unicolor* Latreille, 1804 in Madagascar. Our present study focuses on the western African honeybee *A. m. adansonii* in its local range in Nigeria. Besides Nigeria, *A. m. adansonii* is also indigenous to Burkina Faso and Congo (Engel 1999; Boardman et al., 2019). It is a highly protective species with a high swarming rate (Fletcher 1978; Romet 2001). Presently, *A. m. adansonii* is the only subspecies of *A. mellifera* in Nigeria. It is found across all the eco-geographic regions in Nigeria, ranging from forest and savannah ecosystems (Fasasi et al., 2011; Oyerinde 2017). The traditional beekeepers primarily domesticate it in local artificial hives for honey and beeswax production (Ayoade 1977; Malaka & Fasasi 2005). Morphometry has contributed significantly to improving knowledge of its diversity in Nigeria (Oyerinde et al., 2012a, b). Oyerinde (2017) revealed varying and interrelated morphometric features and wing landmarks between races of *A. m. adansonii* from two vegetation zones in Nigeria. However, little is known about its genetic diversity across the eco-geographic zones in Nigeria.

Incorporating molecular dataset has enabled the understanding of the genetic diversity pattern, biogeography, phylogenetics, evolution and population genetics of insects (Susanta 2006; Andrade-Souza et al., 2017). In particular, analyses of the mitochondrial DNA genes have shown to be an efficient method of determining species' phylogeography owing to its relatively rapid evolution rate and lack of recombination (Moore 1995; Oliveira et al., 2008; Kaltenpoth et al., 2012). Of these mitochondrial genes, subunit 1 of the *cytochrome c oxidase* (COI) has been vastly employed in species identification, reconstruction of matrilineal genealogies, and investigating phylogeography (Hebert et al., 2003; Wang et al., 2014; Nneji et al., 2020). This approach, commonly referred to as DNA barcoding, has aided in the rapid identification of species and analyses of diversity patterns in diverse groups of insects (Andrade-Souza et al., 2017; Nneji et al., 2020a, b). Using the COI gene, past research (such as Pramual et al., 2005; Patel & Jadhav 2019 etc.) have provided valuable information for understanding the population genetic structure and variations within insects. Thus, in conjunction with other mitochondrial and nuclear markers, this method has opened new perspectives for species identification and studies on the genetic diversification of insects (Low et al., 2016; Pramual & Nanork 2012).

Several studies have used different mitochondrial gene markers to establish patterns of population differentiation within various subspecies of *A. mellifera* (Cornuet & Garnery 1991; Arias & Sheppard 1996; Franck et al., 2000; Palmer et al., 2000; Tan et al., 2011; El-Niweiri & Moritz 2008; Ivanova et al., 2011; Szalanski & Magnus, 2010; Alburaki et al., 2013; Achou et al., 2015; Chávez-Galarza et al., 2017; Coulibaly et al., 2019 etc.). Compared to other groups of *A. mellifera*, only a few phylogeographic studies have been carried out on the African group (e.g. Arias & Sheppard, 1996; Franck et al., 2001; Boardman et al., 2019, e.t.c). The previous studies have revealed genetic relatedness of *A. m. adansonii* with other subspecies of African honeybees (*A. m. scutellata*, *A. m. monticola*, and *A. m. capensis*) (Arias & Sheppard 1996; Franck et al., 2001; Boardman et al., 2019). To date, the geographic boundaries of these subspecies remain debatable. In Nigeria, there are fewer to no studies on the characterization and genetic diversity of *A. m.adansonii* using molecular datasets. Most studies focused on the morphology, socio-economic importance of beekeeping, management and/or biology of *A. m. adansonii* (Malaka & Fasasi 2005; Fasasi et al., 2011; Fasasi et al., 2011; Oyerinde 2017 etc.). Thus, to fully comprehend the diversity of *A. m. adansonii* in Nigeria, integrated approaches based on morphology and molecular genetics are required.

In this study, we investigated the population genetic diversity of *A. m. adansonii* in its local range in Nigeria. We sequenced the *cytochrome c oxidase subunit 1* gene (COI) of individuals of *A. m. adansonii* collected from different eco-geographic regions in Nigeria to address this. The objectives of this study were to use the DNA (COI) barcode data to 1) reliably identify and characterize *A. m. adansonii* from Nigeria and 2) evaluate the mitochondrial genetic diversity pattern, population structure, and historical demography of *A. m. adansonii* in Nigeria. This study advances knowledge on the population genetics of Western African honeybee in Nigeria. It provides a valuable theoretical basis for the creation of effective management plans for this economically important insect.

2. Materials and methods

2.1. Study area

Nigeria (Fig. 1) is a country in West Africa. It borders the Gulf of Guinea between Benin (west) and Cameroon (east), and it is located between latitudes 4° and 14° North and longitudes 3° and 15° East (FMoE. 2001). It is an Afrotropical region that occupies a landmass of approximately 932,768 km^2 (FMoE, 2010). The valleys of the Niger and Benue rivers form Nigeria's most extensive topographical region (Fig. 1). The country is rich in natural resources owing to its unique geographic location and eco-climatic variability (Ejidike & Ajayi 2013). There are several bioregions in Nigeria based on topography, climate, rainfall, vegetation, and soil conditions (Iloeje 2001). Nigerian ecological zones are broadly classified into forest and savannah with differences in temperature and precipitation (Fig. 1). For instance, the humid tropical forests of western and southern Nigeria have a more extended rainy season with lower temperatures (Nneji et al., 2019). In contrast, the grasslands (savannah) found mainly in northern Nigeria is characterized by a shorter rainy season and higher temperatures (Nneji et al., 2019). Furthermore, forest clearing for agricultural purposes and urbanization has resulted in grassland with few trees in some western areas in Nigeria. Nevertheless, Nigeria is endowed with diverse plants and animal species. More than 22,000 vertebrate and invertebrate species have been reported, and of these, about 0.14% is threatened, and 0.22% is endangered (FMoE, 2010).

2.2. Taxon sampling, identification, and DNA extraction

Between 2016 and 2018, 14 colonies were sampled from eight states distributed across different eco-geographic zones in Nigeria. A total of two hundred and fourteen (214) adult workers were collected (Table 1) as follows: Cross River ($n = 3$), Enugu ($n = 25$), Kaduna ($n = 12$), Kwara ($n = 34$), Nasarawa ($n = 25$), Ogun ($n = 29$), Osun ($n = 45$) and Oyo ($n = 41$). Details of the locations and the

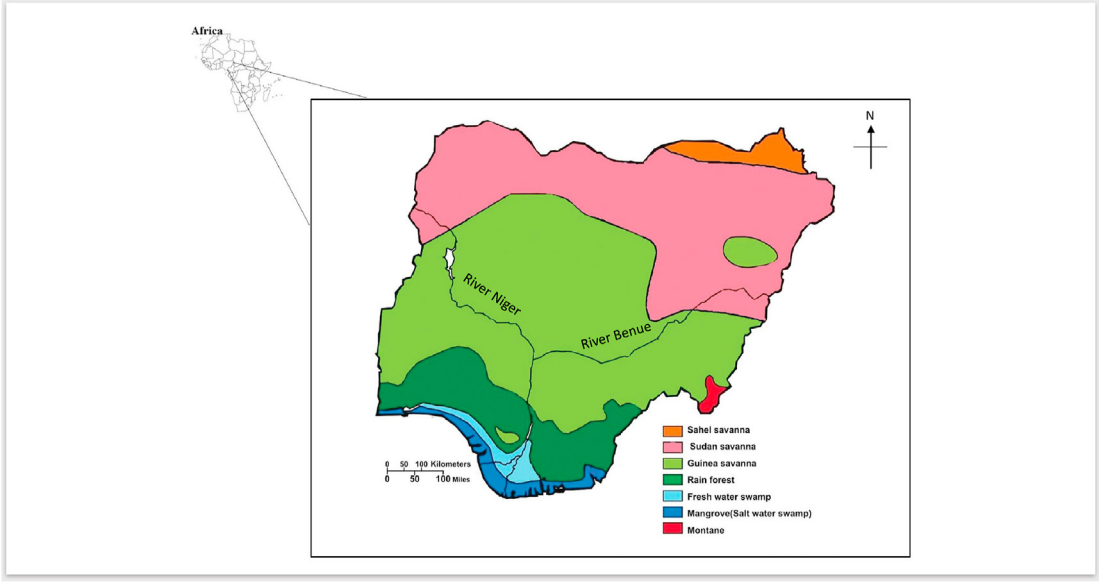


Fig. 1. Map of Nigeria showing the different ecological bioregions; adapted from Nneji et al., 2019.

Table 1
Locality information, including GenBank Accession Numbers, for newly collected *Apis mellifera adansonii* from Nigeria.

S/NO	State	Locality	Latitude	Longitude	Voucher number	Number of samples collected	Genbank accession number
1	Cross River	Calabar	5.013889	8.351110	ASM196-ASM228	3	MT954228- MT954230
2	Enugu	Enugu	6.858056	7.625833	ASM343-ASM367	25	MT954305-MT954329
3	Kaduna	Kaduna	11.066667	7.7003	ASM244- ASM267	12	MT954231- MT954242
4	Kwara						
		Buari	8.477722	4.937167	ASM034 - ASM043	5	MT954140- MT954144
		Malete	8.715972	4.476222	ASM076 - ASM099	15	MT954161- MT954175
		Ilorin	8.567790	4.551840	ASM126 - ASM136	8	MT954176- MT954183
		Afon	8.309911	4.511080	ASM138 - ASM143	6	MT954184- MT954189
5	Nasarawa	Orozo	8.311667	7.916111	ASM318 - ASM342	25	MT954280- MT954304
6	Ogun	Agbungburu	7.186556	3.520444	ASM051 - ASM075	16	MT954145- MT954160
		Omiseeni	7.218778	3.501528	ASM295 - ASM317	13	MT954267-MT954278
7	Osun	Ikirun	7.911320	4.644310	ASM144 - ASM168	21	MT954190- MT954210
		Oshogbo	7.80774	4.56113	ASM268 - ASM292	24	MT954243-MT954266
8	Oyo	Igbeti	8.86629	4.04304	ASM001 - ASM025	41	MT954116- MT954139
Total						214	

numbers of samples are provided in Table 1. The adult workers were collected in the early morning from the entrance of the hive. Individuals were preserved in 99% ethanol and deposited in the Museum of the Department of Biosciences and Biotechnology, Kwara State University, Malete, Nigeria. Morphological identification of the bee was carried out by AMA.

A third right leg was removed from each specimen with sterile forceps and preserved in a tube containing 95% ethanol for the genetic analysis. The ethanol-preserved tissue samples were stored at −80 °C. The protocols for the DNA extraction follow the standard phenol-chloroform method (Sambrook et al., 1989). After extraction, the total genomic DNA samples were preserved in TE buffer, quantified, and further reconstituted for the polymerase chain reaction (PCR).

2.3. PCR amplification and sequencing of the mitochondrial COI gene

Partial sequences of the mitochondrial COI-5′ barcode region were amplified and sequenced for all individuals. The PCR amplification was carried out with a primer pair (LepF1 and LepR1) designed by Hebert et al. (2004). The cycle profiles for the PCR were as follows: initial denaturation of 5 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, annealing for 45 s at 46 °C, an initial extension for 1 min at 72 °C; final extension for 10 min at 72 °C. The resulting PCR products were electrophoresed using 1.2% agarose gel containing gel red. Purified PCR products were directly sequenced bidirectionally with an automated DNA sequencer (ABI 3730 xl).

2.4. Sequence alignment, molecular-based identification, and data assembly

The nucleotide sequences were verified manually using SeqManTMII (DNASTAR Lasergene 7), and contigs were assembled after closely inspecting sequence chromatograms for heterogeneity. The alignment was done utilizing the ClustalW in MEGA 6.0 (Tamura et al., 2013). The nucleotide sequences were translated into amino acids using the invertebrate mitochondrial code. This was to ensure no premature stop codons and confirm that the open reading frame was retained in the protein-coding locus. The identity of the amplified sequences was confirmed by BLAST searching of the newly amplified COI barcode sequences in the Barcode of Life Database (BOLD).

We searched the BOLD database for the previously published COI-5′ barcode region of *A. m. adansonii* from Nigeria. By doing so, thirty-nine (39) COI sequences were retrieved (see Table S1). We combined both the newly amplified and previously published sequences in MEGA and aligned them using ClustalW.

2.5. Mitochondrial genetic diversity and population structure

In this study, we delimited the populations of *A. m. adansonii* in Nigeria into the following subdivisions for the investigation of the mitochondrial genetic diversity and population structure across Nigeria and its eco-geographic regions:

i. The overall population of all samples used in this study,
ii. Grouping was presumed to constitute subpopulations based on geographic distance (northern, southern/eastern, middle belt/central and western Nigeria) and barriers (with respect to the Rivers Niger and Benue). We followed the subdivisions of Iloeje (2001) and Nneji et al. (2019) as follows: western Nigeria (includes populations from Oyo, Ogun, and Osun States); southern Nigeria (comprises individuals from Imo, Enugu, and Cross River States); middle belt Nigeria (consists of populations from Nasarawa, Kwara, and Niger States) and northern Nigeria (includes individuals from Kaduna and Gombe States). These regions have contrasting environmental conditions (Nneji et al., 2019). For instance, while western and southern Nigeria are mainly humid forest, the middle-belt and northern Nigeria are savannah.

Variables including the number of polymorphic/segregating sites (*S*), number of haplotypes (*H*), nucleotide diversity (π), theta (per site) from Eta (total number of mutations) (θ), haplotype diversity (*Hd*), and mean the number of pairwise differences (*k*) were estimated. The indices were calculated in DnaSP v5.10 (Librado & Rozas 2009). We assessed the pairwise evolutionary distances between the geographic and ecological regions using MEGA 6.0. Also, the pairwise Fst values between the geographic regions were evaluated in ARLEQUIN v3.5 (Excoffier & Lischer 2010). An Fst value of 0 means there is no differentiation between subpopulations, while a value of 1 implies clear differentiation (Bird et al., 2017). The Analysis of Molecular Variance (AMOVA; Excoffier et al., 1992) was used to examine population genetic structuring within *A. m. adansonii*. Analyses were performed in ARLEQUIN, and 10,000 random permutations assessed the level of significance. Further, we performed a Mantel test to assess whether genetic differences between populations were attributable to isolation-by-distance. The analysis was conducted in the IBD Web Service (IBDWS; Jensen et al., 2005). The level of significance was assessed using 10,000 matrix permutations.

2.6. Historical demography

We used various statistics to examine historical demographics following subdivisions in section 2.5. For the neutrality test, the values of Tajima's D, Fu and Li's D, and F, and *Fs* were calculated. If population size remains constant, we expect that the values of Tajima's D and Fu and Li's D would be near zero. Also, we presumed that a positive and significant value of Tajima's D and Fu and Li's D would be predicted in population that had recently undergone bottlenecks, while negative and significant values would suggest recent population expansion (Tajima 1989; Fu & Li 1993). For the *Fs* statistic, we presumed that a negative value would indicate recent population expansion or genetic hitchhiking, while a positive value implies a population that had recently undergone bottlenecks (Fu 1997).

Further, we examined the patterns of mismatch distributions to examine if there is any signal of population expansion (Rogers & Harpending 1992). The generalized least square approach was used to compare the nucleotide sequence variations between haplotypes under spatial and demographic models (Excoffier 2004; Excoffier & Lischer 2010). According to Slatkin & Hudson (1991), the multimodal distribution would be expected if a population has been stable over time, while a unimodal pattern would be observed in the case of recent population expansion. The sum of squared deviations (SSD) was used as goodness-of-fit statistics for the mismatch distributions, with the level of significance and confidence intervals assessed using 1, 000 bootstrap replicates. The neutrality test and mismatch distribution were calculated using ARLEQUIN.

2.7. Matrilineal genealogy

Analysis of the matrilineal genealogy involved 253 COI sequences of *A. m. adansonii* comprising 214 newly generated sequences and 39 others downloaded from the BOLD. We downloaded a COI sequence of a closely related species- *Apis cerana* (KJ755584), as the primary outgroup taxon. Using the Akaike information criterion (AIC) performed in MrModelTest 3.7 (Posada & Buckley 2004), the best-fit nucleotide substitution model was determined. A maximum likelihood (ML) approach, as implemented in the RAxML version 7.2.8 (Stamatakis, 2006), was used to infer the matrilineal genealogy. A gamma distribution evolutionary model was selected with 100 random addition replicates and lengths per partition branch. The bootstrap support was assessed using 1000 bootstrap replications. Analyses were performed in the CIPRES Science Gateway version 3.3 web service (Miller et al., 2012). The resulting tree was viewed in FigTree v1.4.2 (Rambaut 2012). Further, we constructed a haplotype network to envisage the associations between haplotypes. To do this, we used the median-joining algorithm (Bandelt et al., 1999), as implemented in Network 5.0.1.1 (www.fluxus-engineering.com).

3. Results

3.1. Sequence information

A total of 634 bp of COI sequence data was aligned. These sites consist of 629 conserved positions and 13 variable regions, of which 10 were parsimony informative. The average contents of Adenine, Thymine, Cytosine, and Guanine were 33.70%, 40.10%, 15.50%, and 10.70%, respectively. The average content of A + T was 73.80%. De novo sequences were deposited in GenBank with numbers MT954116 - MT954329 (Table 1).

Table 2
Summary of the population genetic polymorphism of *A. m. adansonii* based on mtDNA COI gene.

Population Subdivision	S	*h*	*Hd*	π	θ	*k*
Total Population	7	8	0.702	0.00314	0.00378	0.951
Geographic Region						
(i) West	4	6	0.669	0.00290	0.00244	0.87797
(ii) South	2	3	0.440	0.00153	0.00156	0.4640
(iii) Middle belt	6	7	0.748	0.00364	0.00406	1.102
(iv) North	2	3	0.582	0.00221	0.00208	0.670

S: number of polymorphic/segregating sites; *h* = number of haplotypes; *Hd* = haplotype diversity; π = nucleotide diversity; θ = theta (per site) from Eta (total number of mutations); *k* = mean the number of pairwise differences.

3.2. Mitochondrial genetic diversity and genetic structure

For the complete COI dataset of *A. m. adansonii*, a total of eight (8) unique haplotypes were observed. The overall haplotype (gene) diversity was 0.702 (Table 2). Overall, the nucleotide diversity was low ($\pi = 0.00314$), with mean number of pairwise differences of $k = 0.951$ and theta (per site) from eta (θ) = 0.00378 (Table 2). Based on the geographic regions, our data showed that the number of haplotypes for each population ranged from three to seven (Table 2). Populations from middle belt of Nigeria showed the highest number of haplotypes ($h = 8$) and highest values of haplotype diversity ($Hd = 0.748$) nucleotide diversity ($\pi = 0.00364$), mean number of pairwise differences ($k = 1.102$) and theta (per site) from eta ($\theta = 0.00378$).

Extremely low uncorrected *p*-distances (0.00216–0.380%) and pairwise Fst (0.00232–0.19195) between geographic regions were observed (Table 3). When the geographic regions were compared, the mtDNA divergence between the middle belt and western Nigeria was the farthest (p = 0.380%; pairwise Fst value = 0.19195; Table 3). A geographically based hierarchical AMOVA analysis showed that most of the molecular variation was allocated within populations (68.10%), with 39.51% accounting for the variation among populations within groups (Table 4). This was supported by a low genetic differentiation index between groups ($\Phi ST = 0.31986$; Table 4). Our result showed that isolation-by-distance was not evident (correlation coefficient (r) = 0.148, P > 0.001). This result, therefore, indicates no correlation between mtDNA genetic and geographic distances.

3.3. Historical demography

The mismatch distribution (Fig. 2) and neutrality tests (Table 5) supported the hypothesis of population expansion of *A. m. adansonii* in Nigeria. The neutrality test values showed negative values that reached a non-significant level (P > 0.05, Table 5) for populations of *A. m. adansonii* from western and middle belt Nigeria. Also, the SSD values and Raggedness index mismatch analyses were statistically non-significant (P > 0.05, Fig. 2). This implied that the population expansion of *A. m. adansonii* in the western and middle belt of Nigeria should not be ruled out. In contrast, the neutrality tests and mismatch distribution results showed the possibility of a recent bottleneck for the populations from southern and northern Nigeria (Fig. 2, Table 5).

Table 3
Genetic distances between populations of *A. m. adansonii* collected from different geographic regions in Nigeria.

Geographic Region	West	South	Middle Belt	North
West	*	0.048	0.19195	0.00232
South	0.234	*	0.14718	0.16905
Middle Belt	0.380	0.00334	*	0.13501
North	0.257	0.00216	0.00367	*

Note: Top of the diagonal is the population pairwise Fst values; below the diagonal is the uncorrected p-distances (expressed in percentage).

3.4. Matrilineal genealogy

The ML analysis (Fig. S1) obtained a single matriline for *A. m. adansonii* in Nigeria. No genealogical clusters corresponding to either sampling sites or geographic regions were shown on the ML phylogenetic tree (Fig. S1). The median-joining network of the COI gene revealed a lack of clear population differentiation (Fig. 3). These results are consistent with the historical demographics indicating a population expansion event occurring after a bottleneck for *A. m. adansonii* in Nigeria.

4. Discussion

In this study, the mitochondrial COI gene was used for the identification and analysis of the genetic diversity and population structure of the western African honeybee *A. m. adansonii* in Nigeria. To the best of our knowledge, our study is the first to examine the mitochondrial genetic diversity of *A. m. adansonii* in Nigeria using the COI gene. We highlighted a high mitochondrial genetic diversity and no evidence of population structuring despite the contrasting environments (ecological regions) between the geographic regions (West, South, Middle Belt, and North) where *A. m. adansonii* were collected. On the African continent, the genetic diversity of honeybees is still understudied due to the lack of reference DNA sequence data for genetic studies (Dietemann et al., 2009; Coulibaly et al., 2019). This makes the conservation of genetic resources incredibly challenging. Our study contributes genetic (DNA barcode) dataset and also provides important insights into the population genetics of an economically important insect species in the region. This information could contribute towards monitoring the native species, enhancing policies for the management of honeybees, and designing strategies for the conservation of the local population. Also, the genetic approach used in our study can be extended in studying the population genetics of other insect species in Africa, for improved and sustainable conservation and management policies.

Characterizing the pattern of genetic variation at intra- and inter-population levels is a fundamental prerequisite for the development of programs aimed at genetic resource conservation. Our result showed that high mitochondrial genetic diversity has been retained for *A. m. adansonii* in Nigeria. Despite our use of the mitochondrial COI gene, we obtained mitochondrial haplotype diversity similar to and in some cases, higher than those recorded for several other African honeybee populations (Franck et al., 1998;

Table 4
Historical demography of *A. m. adansonii* from Nigeria based on mtDNA COI gene.

Population Subdivision	Tajima's D	Fu and Li's D	Fu and Li's F	Fu's Fs	SSD	Raggedness Index
Total Population	−0.35075*	−0.82850*	−0.77391*	−1.322*	0.02124	0.08681
Geographic Region						
(i) West	0.35742*	−0.31472*	−0.11302*	−0.583*	0.05605	0.21557
(ii) South	0.03541*	0.77411*	0.62523*	0.078*	0.08160	0.40500
(iii) Middle belt	−0.24791*	−1.60652*	−1.36964*	−1.184*	0.01211	0.03984
(iv) North	0.17874*	−0.44573*	−0.32441*	0.055*	0.11233	0.41082

Note.
SSD; sum of squared deviations.
*Statistical significance; Not significant, P > 0.05.

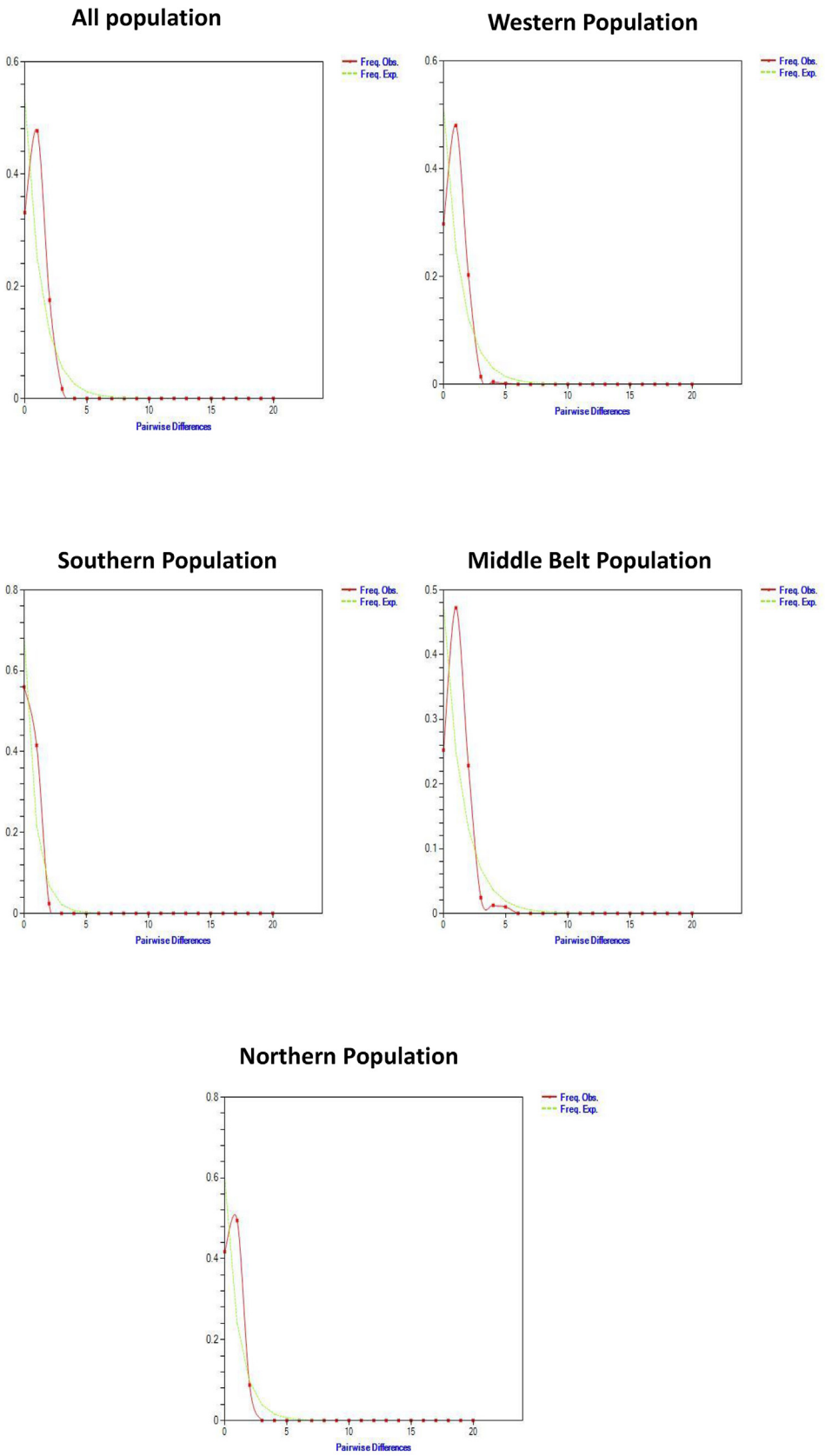


Fig. 2. Mismatch distribution of *Apis mellifera adansonii* based on mtDNA COI gene.

Table 5
The Analysis of Molecular Variance (AMOVA) of the populations of *A. m. adansonii* based on mtDNA COI gene.

Source of variation	Degree of freedom	Sum of squares	Percentage of variation	Fixation Index (ΦST)
Among groups	3	14.245	−7.52	
Among populations within groups	7	52.296	39.51	0.31986
Within populations	242	135.135	68.01	
Total	252	201.676		

Franck et al., 2001; De la Rúa et al., 2007; Achou et al., 2015). The presence of high mitochondrial genetic diversity of *A. m. adansonii* could reflect long-term established populations in Nigeria. It is evident that over time, *A. m. adansonii* accumulated high levels of mitochondrial genetic diversity, countering the effects of any initial demographic bottleneck. Our study, therefore, shows that the genetic diversity of *A. m. adansonii* in Nigeria is abundant, which is conducive for the maximization of the species' germplasm resources and effective beekeeping programs. However, because our analyses involved one locus of the mitochondrial gene of *A. m. adansonii*, the genetic diversity pattern should be taken with caution. Further study involving diverse genetic markers, including mitochondrial and nuclear genes, can unveil the true picture of the genetic diversity of *A. m. adansonii* in Nigeria.

Worth mentioning is the genetic diversity of *A. m. adansonii* observed for populations from southern and northern Nigeria. The low genetic diversity, and evidence from the neutrality tests and population demographics, reveal that *A. m. adansonii* from southern and northern Nigeria may have experienced population or genetic bottleneck. A similar result was obtained for the African honeybee populations from Guinea and northern Morocco (Franck et al., 1998). It is worth noting that the sample sizes of *A. m. adansonii* are not equal among the groupings in our study and this may have an effect in the diversity measures. Hence, more elaborate studies involving relatively uniform population size will uncover the mitochondrial genetic diversity of *A. m. adansonii* from southern and northern Nigeria.

The result of the matrilineal genealogy, median-joining network analysis and the molecular AMOVA revealed an absence of population structure despite a high genetic diversity and significant variability of environmental conditions between the geographic regions examined. The simple topology of the ML genealogy showed no significant lineage branches corresponding to the geographic regions. Low population structuring has been reported in different subspecies of *A. mellifera.* For instance, Ivanova et al. (2010) detected low levels of mitochondrial genetic differentiation in a subspecies of *A. mellifera* from Bulgaria. Contrastingly, previous investigations have highlighted high genetic differentiation related to ecological factors between populations of other subspecies of *A. mellifera* in Africa (El-Niweiri & Moritz 2008; Coulibaly et al., 2019). Apart from the choice of molecular markers used, the difference between these previous studies (El-Niweiri & Moritz 2008; Coulibaly et al., 2019) and ours could be explained by the fact that the honeybee populations examined by these previous studies might have been adapted to the different environmental conditions in the study areas. Indeed, the absence of geographic structuring, despite the contrasting ecological conditions, could suggest human-assisted dispersal and this factor may have played a vital role in the diversification of *A. m. adansonii* in Nigeria. This hypothesis awaits further testing by using multiple

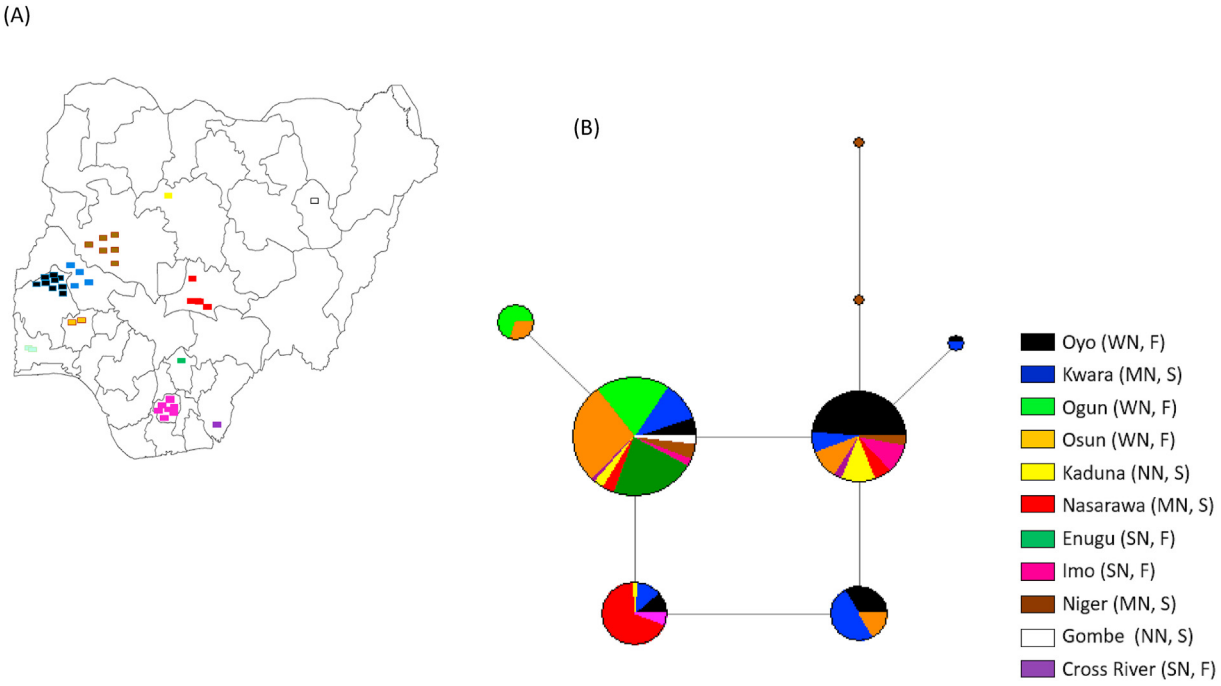


Fig. 3. Median-joining network of mitochondrial *Cytochrome c oxidase subunit I* gene of *Apis mellifera adansonii* in Nigeria. A circle represents each haplotype. Relative sizes of the circles indicate haplotype frequency. Circles of the same color represent haplotypes from the same population. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

mitochondrial and nuclear loci and inclusion of samples of *A. m. adansonii* from other states in Nigeria.

The current study focused on *A. m. adansonii* in its local range in Nigeria. Further studies that cut across its local range distribution in Nigeria, Burkina Faso and Congo would shed more light on genetic diversity and structuring among Western African honeybee populations. Furthermore, although we have reported the estimations of mitochondrial genetic diversity of *A. m. adansonii* in Nigeria, further validation of these estimates is required using multiple gene loci.

5. Conclusion

We observed a high mitochondrial genetic diversity in *A. m. adansonii* populations from Nigeria based on the COI dataset. The absence of population genetic structure represents vital information for the management of honeybees in Nigeria. We recommend holistic approaches that would include morphology, behaviour, ecology, and genetic dataset, for a comprehensive study on the genetic diversification and best management policies for *A. m. adansonii* in its local range in West Africa. Further studies involving additional mitochondrial and nuclear gene markers would help to elucidate patterns of gene flow among populations of *A. m. adansonii* across its range in West Africa. Such studies will also give deep insight into the evolution of *A. m. adansonii*. It will further set the base for further genetic studies that would help design effective management policies for sustainable beekeeping in Nigeria and West Africa.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcz.2021.05.007.

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