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Molecular characterization and phylogenetic analysis of collected mosquitoes (Diptera: Culicidae) from Northcentral Nigeria using mitochondrial *COI* and ribosomal *IGS* gene regions

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Abstract

Background: Mosquitoes are important vectors of disease-causing organisms such as filarial worms, malaria parasites, and arboviruses endemic to sub-Saharan Africa including Nigeria. Malaria is a disease caused by a plasmodium parasite, transmitted by the bite of infected mosquitoes and is no doubt a public health concern. There is limited information on the genetic diversity of mosquitoes in Nigeria. This is necessary because information about the genetic diversity of mosquitoes is a very important step towards vector control and management with the aim to mitigate or eliminate burden resulting from malaria and other diseases caused by mosquitoes. In the present study, we investigated the genetic variability and relatedness of mosquitoes based on the DNA sequences of the mitochondrial cytochrome oxidase subunit I (*COI*) and ribosomal intergenic non-coding spacer gene regions (*IGS*). Mosquitoes were collected from five different states in Northcentral Nigeria, they were morphologically identified using standard keys and genomic DNA was extracted. The specific regions of interests were amplified, and the PCR products were then sequenced.

Results: PCR was able to successfully amplify the expected amplicon sizes of *COI* and *IGS* sequences (710 and 169) base pairs, respectively. For *COI* sequence, pairwise genetic distance between mosquito species ranged from 0.00 to 0.17 in the *COI* sequences. The pairwise genetic distance among *Culex*, *Aedes* and *Anopheles* species in the *IGS* sequences ranged from 0.000 to 0.118. Phylogenetic analysis of the sequences of the mitochondrial cytochrome oxidase subunit showed that there was genetic diversity amongst the different mosquito species sampled. It effectively showed marked differences between *Culicine* and *Anopheline* mosquitoes.

Conclusions: The ribosomal *IGS* primers used for this study only amplified Anopheles spp. However, it revealed that there is diversity among the *Anopheles gambiae* and *Anopheles arabiensis* samples collected. This study concludes that the mitochondrial *COI* and ribosomal *IGS* gene regions are reliable markers for mosquito genetic diversity study and will surely yield a reliable result for molecular diversity assessment of mosquito species.

Keywords: Mosquitoes, Cytochrome oxidase subunit I, Intergenic spacer region, Genetic diversity

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Background

Mosquitoes belong to the family Culicidae which is comprised of three sub families which include *Toxorhynchitinae*, *Anophelinae* and *Culicinae*. *Toxorhynchitinae,* which has only one genus *Toxorhynchites,* is not of any medical importance because it feeds on nectar rather than blood (Collins & Blackwell, 2000). Mosquitoes serve as major vectors of several diseases because it feeds on blood and transmits viral, bacterial, protozoan and other diseases (Becker et al., 2010). Mosquitoes occur in a wide range of both aquatic and terrestrial habitats and have different morphological and behavioural adaptations to these habitats (Becker et al., 2010). Culicine mosquitoes like *Aedes* spp. and *Culex* spp. are important carriers of human pathogens (Molina-Cruz et al., 2013). Anopheline mosquito is the major vector of human malaria. Identifications of mosquito species are based on main morphological features such as legs, antennae, wings and position of the abdomen. However, many mosquitoes exist as species complexes, such as *Culex pipiens* L. *sensulato* (s.l.) (Cornel et al., 2012) and *Anopheles gambiae* s.l. (Scott et al., 1993), limiting their identification based on morphology alone. Previous mosquito diversity studies in Nigeria have mainly employed morphology (Afolabi et al., 2019; Oduola et al., 2013) with few studies on molecular analysis to identify the different mosquito species (Lamidi et al., 2017; Msugh-Ter et al., 2017).

Molecular approaches to support morphological data provide better insight into the genetic diversity of mosquito species (Kumar et al., 2007) and may play a vital role designing and executing effective strategies in the malaria vector control strategies. Different molecular markers have been used for characterization, diversity and population genetic analysis of mosquitoes. Mitochondrial DNA analysis has proven to be a useful and reliable marker for population genetics, intraspecific phylogeography, and systematics (Zink & Barrowclough, 2008). Mosquitoes have been successfully differentiated genetically using the mitochondrial cytochrome c oxidase I (*COI*) gene region (Hebert et al., 2003). Krüger et al. (2014) have previously used *COI* gene region for molecular identification of *Aedes geniculatus*. *Anopheles daciae* mosquitoes have characterized in Germany using *ITS2* sequence analysis (Weitzel et al., 2012). Genetic diversity and molecular characterization of mosquitoes in North-Central Nigeria using ribosomal DNA *ITS2* and mitochondrial *16S-DNA* sequences have been reported (Iyiola et al., 2020). The *IGS* region is a reliable molecular marker in studying genetic and phylogenetic divergence between closely-related of Anopheles mosquito species (Sharma & Chaudhry, 2010).

Variations in larval environment may contribute to insecticide susceptibility of Anopheles mosquitoes (Owusu et al., 2017). Taken into consideration genetic modifications that can be induced due environmental influences, the high abundance and widespread presence of invasive species reported across the world, there is a need to provide a baseline information on genetic diversity of mosquitoes in North-central Nigeria. This is vital because precise, consistent and reliable identification of mosquitoes using DNA-based approach is important and will go a long way to provide a baseline data required in malaria vector control strategies and war against malaria vector in Nigeria. Our central hypothesis is that due to constant genetic mutation, there could be invasive mosquito species in Nigeria which could be a setback to sustainable approaches for surveillance, prevention and control of vector-borne diseases. Therefore, this study investigated genetic diversity study of mosquitoes using a molecular approach. We investigated species composition and genetic diversity between and within mosquito species in North-Central Nigeria using the mitochondria *COI* and ribosomal DNA intergenic spacer *IGS* gene regions. Data from this study will provide a robust assessment of mosquito species' distributions and molecular diversity in Northcentral Nigeria.

Materials and methods

Sample collection

Mosquito larvae were collected from different mosquito breeding sites like abandoned earthen ponds, gutters, stagnant water, water storage containers, abandoned car tyres, etc. in five states in North-Central Nigeria. The states include Benue, Kogi, Kwara and Niger States and Abuja, the Federal capital of Nigeria. The samples were collected from April 2018 to March 2019. Samples were transported to the laboratory and separated into different genera based on the presence or absence of breathing tubes and the length of breathing tubes. They were then kept in bowls labelled based on location and genera. Upon emergence into adult, the mosquitoes were collected using aspirator and put in a labelled 1.5 ml Eppendorf tubes and preserved dry on desiccated silica gel for further analysis.

Morphological identification

Morphological identification of mosquitoes collected was carried out with the aid of a dissecting microscope using standard morphological keys as described by Gillies and Coetzee (1987).

DNA extraction

Genomic DNA was extracted from the whole body of the mosquitoes using Zymo Research Insect Mini prep kit with slight modifications to the manufacturer's protocol. The isolated genomic DNA (gDNA) was checked using

NanoDrop 2000c to check for the quantity and purity of the samples. The genomic DNA extracted was also run on 1% agarose prepared with 1X TBE buffer at 80 V for 1 h to confirm the presence of DNA.

PCR amplification of the mitochondrial cytochrome oxidase subunit I (COI) region

The mitochondrial cytochrome oxidase subunit I (*COI*) gene region was amplified using primers stated in Table 1. PCR amplification was carried out using 5X Hot FIREPol Blend Master Mix with 7.5 mM $MgCl_2$ from Solis BioDyne Estonia. A 25 µL reaction mixture was prepared using 5 µL of 5X Hot FIREPol, 0.25 µL each of both primers, 14.5 µL of nuclease free water and 5 µL of template DNA. The PCR was run using the following conditions: initial denaturation at 95 ° for 15 min, denaturation at 95 ° for 30 s, annealing at 42 ° for 1 min, elongation at 72 ° for 1 min for 30 cycles and final elongation at 72 ° for 10 min. 5µL of each reaction mixture was run on 1.8% DNA agarose gel with 1X TBE running buffer and stained with ethidium bromide stain. Electrophoresis was conducted at 80 V, 150 mA for 1 h 30 min. The gel was viewed under a UV transilluminator.

PCR amplification of the intergenic spacer (IGS) region

The primers used for the amplification of this region were adapted from Ajamma et al. (2016a, b). The primers details are provided in Table 1.

PCR amplification was carried out using 5X Hot FIREPol Blend Master Mix with 7.5 mM $MgCl_2$ from Solis BioDyne Estonia. A 25 µL reaction mixture was prepared using 5 µL of 5X Hot FIREPol, 0.25 µL each of both primers, 14.5 µL of nuclease free water and 5 µL of template DNA. The PCR was run using the following conditions: initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 30 s, annealing at 50 °C for 1 min, elongation at 72 °C for 1 min for 30 cycles and final elongation at 72 °C for 5 min. 5µL of each reaction mixture was run on 2% DNA agarose gel with 1X TBE running buffer and stained with ethidium bromide stain. Electrophoresis was conducted at 90 V, 150 mA for 90 min. The gel was viewed under UV light in a gel documentation system.

Phylogenetic analysis

The PCR mixtures were sent to Inqaba Biotech, South Africa for sequencing. Sequences in ABI format was subjected to trimming, editing and base culling were carried out using Sequencher 5.4.6. The sequences were exported to notepad. These text files were aligned using the ClustalW multiple alignment feature on BioEdit and the aligned sequences were saved as FASTA files, later opened on MEGA7 and phylogeny was inferred using maximum likelihood tree with 1000 bootstrap replications.

Results

Species composition

We determined species composition of mosquitoes collected from Niger, Kogi, Benue, Kwara states and Abuja in North-central Nigeria. A total of 2,100 mosquito samples were collected and they included *Culex*, *Anopheles* and *Aedes* species. *Culex* spp. were the most abundant of all the mosquitoes species collected during this study (Table 2). *Aedes* spp. were found in Benue, Kwara and Kogi States. *Anopheles* spp. were the abundant mosquito species collected from Benue State.

Table 2 Number of mosquitoes collected from different sites in North-Central Nigeria

Locations	Mosquitoes			Total (100)
	Anopheles	Culex	Aedes	
Kwara	11	65	24	100 (4.8)
FCT (Abuja)	3	217	–	220 (10.5)
Niger	6	744	–	750 (35.7)
Kogi	–	14	285	299 (14.2)
Benue	600	130	1	731 (34.8)
Total	620	1170	310	2100 (100)

Table 1 Primer sequences from *COI* and *IGS* sequences used for detection of polymorphism in the Mosquito populations

S/No	Gene region	Primer names	Nucleotide sequences (5′–3′)	% GC content	Expected PCR product size (base pairs)	References
1	*COI*	Forward	GGTCAACAAATCATAAAGATATTGG	32.0	710	Folmer et al., (1994)
2	*COI*	Reverse	TAAACTTCAGGGTGACCAAAA AATCA	34.6	710	Folmer et al., (1994)
3	Ribosomal DNA *IGS*	Forward	GTGAAGCTTGGTGCGTGCT	57.9	169	Ajamma et al., (2016a, 2016b)
4	Ribosomal DNA *IGS*	Reverse	GCACGCCGACAAGCTCA	64.7	169	Ajamma et al., (2016a, 2016b)

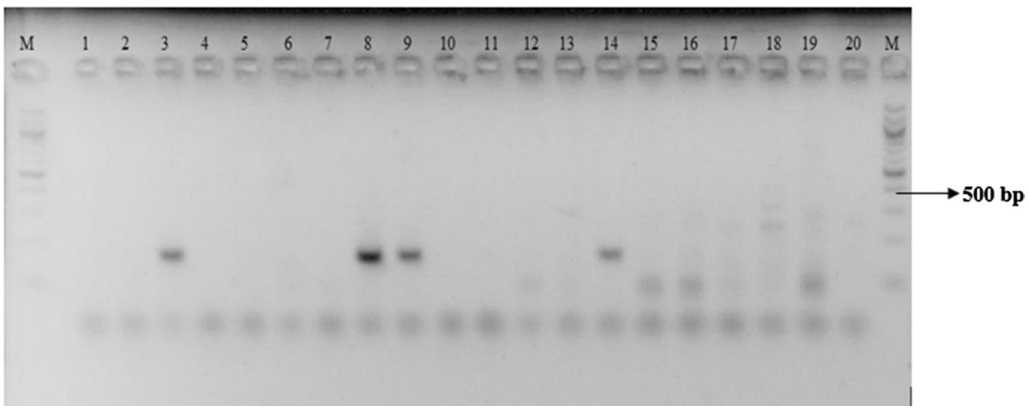

Fig. 1 Amplification profile of mosquito species with primers from the *IGS* gene region. M = DNA markers (size 100 bp), 1–20: Mosquito samples

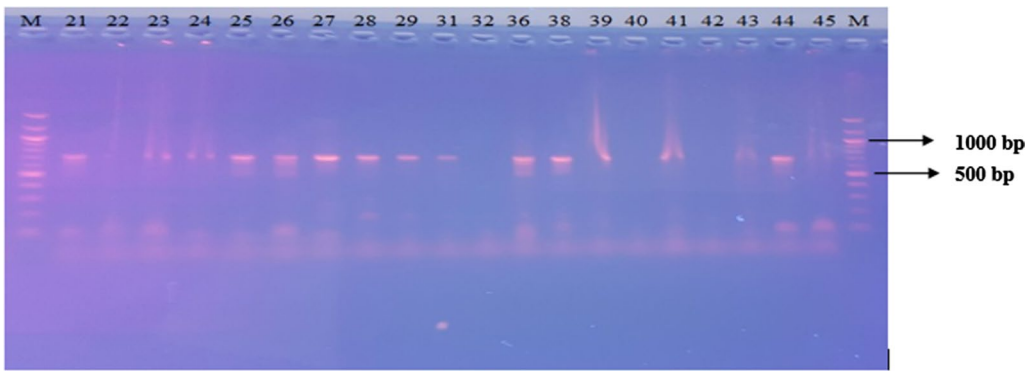

Fig. 2 Amplification profile of mosquito species with primers from the *COI* gene region. M = DNA markers (size 100 bp), 21–45: Mosquito samples

Molecular analysis and PCR amplification

DNA agarose gel images of the PCR amplicons of the mitochondrial *IGS* and *COI* gene regions, respectively, after electrophoresis (Figs. 1, 2). The *IGS* and *COI* gene regions of all the extracted DNA of the mosquito samples were amplified successfully using primers stated in Table 1. PCR mixtures were loaded on 1.8% agarose and run at 90 V for 90 min. It was then viewed with UV transilluminator and photographed. Lane M is the 100 bp DNA ladder, while lane 21–45 is the PCR amplicons of different mosquito samples. The expected and amplified PCR fragment sizes were 169 and 710 (base pairs) for *IGS* and *COI* gene regions, respectively, (Figs. 1 and 2).

Phylogenetic analysis

Dendogram of the intergenic spacer region sequences of the *Anopheles* spp. amplified for this study is shown in Fig. 3. The phylogenetic tree constructed is a Maximum-Likelihood tree with 1000 bootstrap replications according to Tamura-Nei method. Green circles in Fig. 3 represent the samples sequenced in this study and those in red boxes are *Anopheles* spp. sequences retrieved from the NCBI database. *Culex pipiens pipiens* sequence, denoted in blue diamond, also retrieved from the NCBI database was used to represent the outgroup. *Anopheles gambiae* haplotype in this study clustered together with the sequence available at the GenBank database and was isolated from Guinea Bissau (Ascension number KX828857.1). The sequences are supported by a bootstrap value of 68 at the node. Likewise, the *Anopheles arabiensis* also clustered with the sequences gotten from Brazil but was supported by a bootstrap of 65 at the node. (Fig. 3). Dendogram of the mitochondrial cytochrome oxidase subunit I of the mosquito species is shown in Fig. 4. The lemon circles denote the mosquito samples sequenced for this study, the lilac triangles represent the sequences collected from the NCBI database and the red diamond represents the outgroup used to root the tree. Some *Culex pipiens* sequence in this study showed divergence with those from Turkey, while others clustered with the sequences from Kenya. *Culex watti* and *Culex antennatus* sequences from this study also clustered with

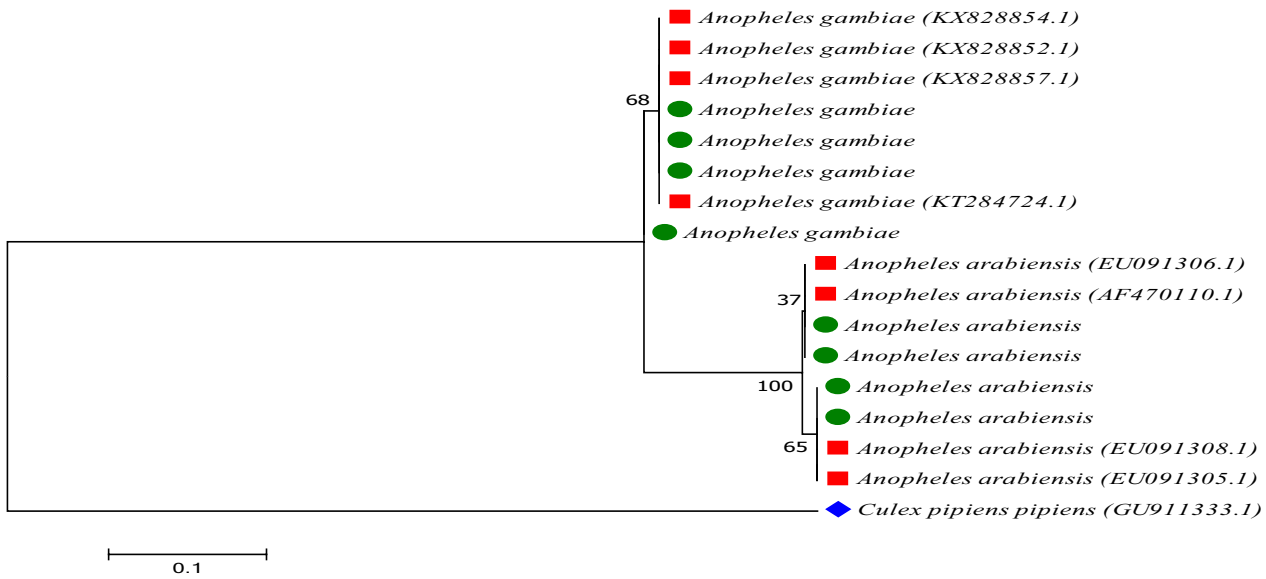

Fig. 3 Molecular phylogenetic analysis of the intergenic spacer (*IGS*) region of *Anopheles* spp. by Maximum Likelihood method inferred by Tamura-Nei method after 1000 replications. *Culex pipiens pipiens* was used as an outlier

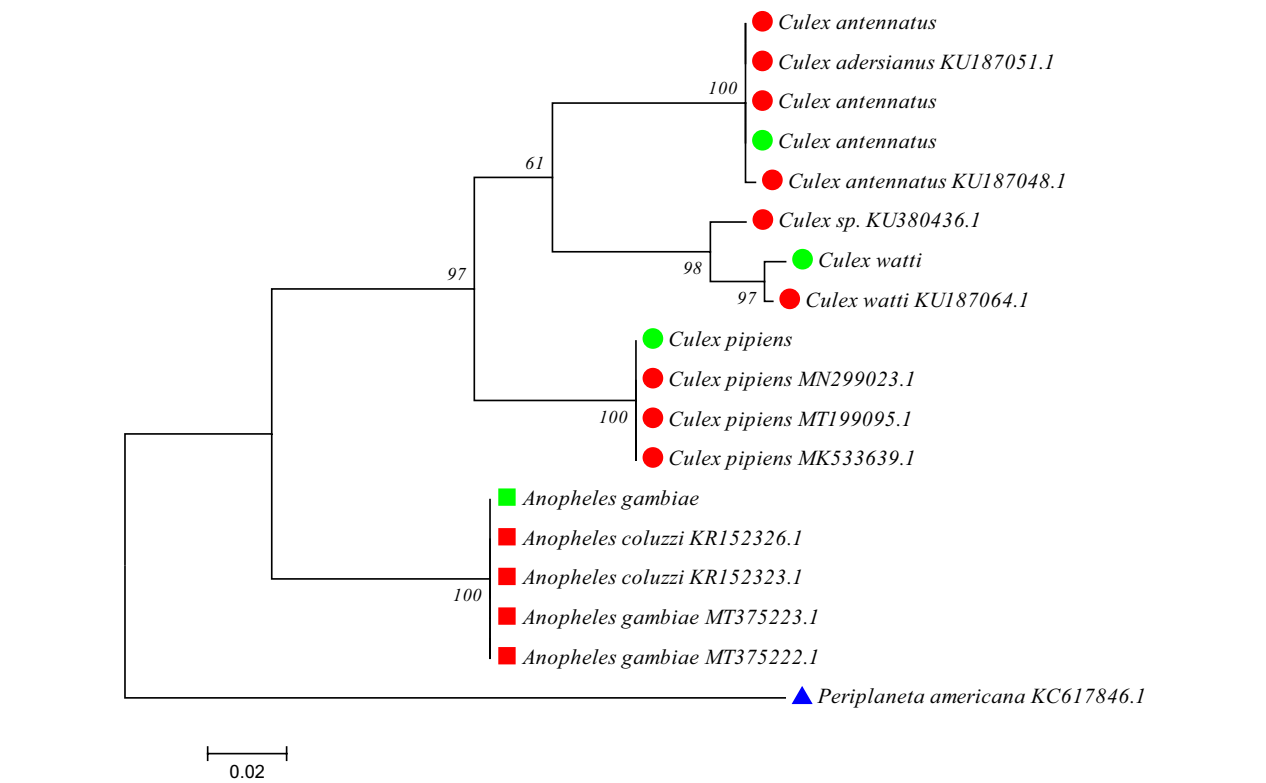

Fig. 4 Molecular phylogenetic analysis of the mitochondrial cytochrome oxidase subunit I (*COI*) region of mosquito species by Maximum Likelihood method inferred by Tamura-Nei method after 1000 replications. *Periplaneta americana* was used as an outlier

same species from Kenya and they are greatly supported with a bootstrap > 90 (Fig. 4). Data from analysis of evolutionary divergence between *COI* and *IGS* gene sequences of some mosquito species used in this study are as indicated in Tables 3 and 4, respectively. DNA nucleotide sequences were deposited at the GenBank database for future reference, and they were assigned with Genbank accession numbers OK257522-OK257529 for *COI* and OK236349-OK236355 for *IGS*.

Estimates of evolutionary divergence between the *COI* sequences obtained from the *Culex a*nd *Anopheles* species are shown in Table 3. As expected between mosquito species, the estimated pairwise genetic distance was observed between *Anopheles gambiae* and *Culex pipiens* species in the *COI* sequences from mitochondrial DNA at 0.17.

In Table 4, the DNA nucleotide sequence of *Anopheles gambiae* collected from Ilorin, Kwara State is genetically different from *Anopheles gambiae* collected from Abuja and Niger state of Nigeria with a pairwise genetic distance value of 0.009. *Anopheles arabiensis* collected from Yandev, Benue State, Nigeria is genetically different within species with a pairwise genetic distance value of 0.009. The highest pairwise genetic distance was 0.118 was observed between *Anopheles gambiae* and *Anopheles arabiensis.*

Discussion

In this study, 2,100 mosquito samples were collected and Anopheles sp has the highest proportion in Benue state with non in Kogi state. *Culex* sp was found to have highest species composition in Niger states. Similarly, Aedes sp was found to have the highest abundance in Kogi state with no Aedes reported in both Niger state and Abuja. The presence of mosquitoes in the studied locations could be due to overpopulation of these areas which may have led to improper waste and sewage disposal which provides a suitable habitat for the *Culex* mosquito species. Mosquitoes' abundance could be related to urbanization, over-abundance of human activities as well as other anthropogenic activities that has led to poor waste disposal, poor sanitary levels, uncontrolled run-offs, etc. which has created a habitat for breeding of these mosquitoes. This result is consistent with data from a study carried out in Benin City by Aigbodion and Uyi (2013) which reported that *Culex* and *Aedes* had higher abundance over *Anopheles* mosquitoes. Mosquitoes collected from Benue states showed a higher relative abundance of *Anopheles* species. It is reasonable to suggest that relative species abundance of *Anopheles* may likely lead to prevalence of malaria especially among infants, pregnant women and immuno-compromised individuals in this area if no vector control measures are put in place. This observation is consistent with the report of a study carried out by Aju-Ameh et al. (2017) where the highest *Anopheles* mosquitoes were recorded in Gboko, Benue state, Nigeria. In Ilorin, Kwara State, three mosquito genera were recorded and *Culex* spp was the most prevalent. However, a study by Ajao and Adeleke (2014) had the highest abundance and this was different from the report from a study conducted by Amaechi et al. (2018), where *Anopheles gambiae* had the highest abundance.

The intergenic spacer (*IGS*) primers used for this study were only able to distinguish between *Anopheles* spp. The intergenic region produced amplicon size of 169 bp. Sequence variations were observed in the DNA based-nucleotide sequence of the *IGS* gene region. Nucleotide bases substitutions of both transition and transversion types were observed when the sequenced DNA was aligned. Twelve (12) variable sites were observed among all the species which included 5 transversion and 7 transitions. Between the *An. gambiae* and *An. arabiensis*, 11 variable sites were observed, with A-G transitions being the most common. A single variable site of G-T and A-T transversion was also observed within the species of *An. gambiae* and *An. arabensis*, respectively. However, for *COI* sequence, we observed that evolutionary individual divergence between species ranged from 0.000 to 0.009 (Table 3). The pairwise genetic distances were determined for *Anopheles* species. Low genetic distance value was observed. This observation indicates that the *Anopheles* diverge more recently. This also showed that there is low individual divergence among the *Anopheles* species of mosquitoes collected in this study. This is consistent with previous study of Hamza et al. (2014) which reported high genetic similarities and low individual divergence within the *An. gambiae* individuals collected from Sudan. From the phylogenetic trees, it was observed that the region could differentiate the different mosquito subfamilies with Anophelinae occupying the basal position. This region could also differentiate the different mosquito species genera which is in correlation with a study of Ajamma et al. (2016a, b) where it was reported that *COI* was able to distinguish between members of *Mansonia*, *Culex* and *Aedes*. The phylogenetic tree constructed from *IGS* gene region is shown in Fig. 3. It identified two *Anopheles* species complex which are *An. gambiae* and *An. arabiensis* and were able to separate between the two species of *Anopheles* as they both form a separate clade on the phylogenetic tree with the species of *An. gambiae* clustering together and that of *An. arabiensis* also clustered together (Fig. 3). This is supported by the study of Ajamma et al. (2016a, b) where it was reported that *IGS* gene region was able to successfully distinguish between Anopheles

Table 3 Estimates of evolutionary divergence between mitochondrial *COI* sequences of some mosquitoes used in this study

S/N	Organism	Source	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	Culex_pipiens_OK236349	This study	0.00																		
2	Culex_antennatus_OK236350	This study	0.10	0.00																	
3	Anopheles_gambiae_OK236351	This study	0.15	0.15	0.00																
4	Culex_antennatus_OK236352	This study	0.10	0.00	0.15	0.00															
5	Culex_pipiens_OK236353	This study	0.00	0.10	0.14	0.10	0.00														
6	Culex_watti_OK236354	This study	0.09	0.09	0.16	0.09	0.09	0.00													
7	Culex_antennatus_OK236355	This study	0.10	0.00	0.15	0.00	0.10	0.09	0.00												
8	Culex_pipiens_MN299023.1	Columbia	0.00	0.10	0.15	0.10	0.00	0.09	0.10	0.00											
9	Culex_pipiens_MT199095.1	Egypt	0.00	0.10	0.15	0.10	0.00	0.09	0.10	0.00	0.00										
10	Culex_pipiens_MK533639.1	Kenya	0.00	0.10	0.15	0.10	0.00	0.10	0.10	0.00	0.00	0.00									
11	Culex_adersianus_KU187051.1	Kenya	0.11	0.01	0.17	0.01	0.10	0.09	0.01	0.11	0.11	0.11	0.00								
12	Culex_antennatus_KU187048.1	Kenya	0.11	0.01	0.17	0.01	0.10	0.09	0.01	0.11	0.11	0.11	0.00	0.00							
13	Culex_watti_KU187064.1	Kenya	0.09	0.09	0.16	0.10	0.08	0.01	0.09	0.09	0.09	0.10	0.10	0.09	0.00						
14	Culex_sp._KU380436.1	Kenya	0.10	0.08	0.17	0.08	0.09	0.03	0.08	0.10	0.10	0.10	0.09	0.09	0.03	0.00					
15	Anopheles_coluzzi_KR152326.1	Togo	0.15	0.15	0.00	0.15	0.14	0.15	0.15	0.15	0.15	0.15	0.16	0.17	0.16	0.17	0.00				
16	Anopheles_coluzzi_KR152323.1	Togo	0.15	0.15	0.00	0.15	0.14	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.15	0.16	0.00	0.00			
17	Anopheles_gambiae_MT375223.1	Kenya	0.15	0.15	0.01	0.15	0.14	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.15	0.16	0.00	0.01	0.00		
18	Anopheles_gambiae_MT375222.1	Kenya	0.15	0.15	0.00	0.16	0.14	0.15	0.15	0.15	0.15	0.15	0.16	0.17	0.15	0.16	0.00	0.00	0.00	0.00	
19	Periplaneta_americana_KC617846.1	USA	0.27	0.28	0.22	0.27	0.27	0.28	0.27	0.27	0.28	0.28	0.29	0.28	0.29	0.30	0.24	0.23	0.22	0.22	0.00

Table 4 Estimates of evolutionary divergence between the *IGS* region sequences of some *Anopheles* species used in this study

Mosquito species	Location	*Anopheles gambiae*	*Anopheles gambiae*	*Anopheles gambiae*	*Anopheles gambiae*	*Anopheles arabiensis*	*Anopheles arabiensis*	*Anopheles arabiensis*	*Anopheles arabiensis*
An. gambiae	Ilorin, Kwara	–							
An. gambiae	Abuja (Kubwa)	0.009	–						
An. gambiae	Abuja (Utako)	0.009	0.000	–					
An. gambiae	Suleja, Niger	0.009	0.000	0.000	–				
An. arabiensis	Benue (Ipav)	0.096	0.107	0.107	0.107	–			
An. arabiensis	Benue (Mbayion)	0.107	0.118	0.118	0.118	0.009	–		
An. arabiensis	Benue (Yandev)	0.107	0.118	0.118	0.118	0.009	0.000	–	
An. arabiensis	Benue (Yandev)	0.096	0.107	0.107	0.107	0.000	0.009	0.009	–

mosquito species. *Anopheles gambiae* haplotype in this study clustered together with the sequence retrieved from GenBank database and was isolated from Guinea Bissau (Ascension number KX828857.1). The sequences are supported by a bootstrap value of 68 at the node. Likewise, the *Anopheles arabiensis* also clustered with the sequences gotten from Brazil but was supported by a bootstrap of 65 at the node. (Fig. 3). This is an evidence of identical and clearly established phylogenetic relationship among the Anopheles species used in this study Guinea Bissau anopheles retrieved from Genbank. The phylogenetic tree in Fig. 4 showed that the different *Culex* species, namely *C. pipiens, C. watti* and *C. antennatus,* formed separate clades of similar species clustering together and supported by a good bootstrap value. *C. pipiens* from this study clustered with *C. pipiens* from Kenya and Turkey, while *C. watti* from this study clustered with the one from Kenya. This revealed evidence of identical and clearly established phylogenetic relationship among the Culex species used in this study due to clustering of similar species on the same nodes. Similarly, An. gambiae from this study clustered with Uganda anopheles retrieved from Genbank database. There is no doubt that these show evidence of similarity in the mtDNA sequence of Culex species from our study and those from Kenya, Turkey and Anopheles. It could be hypothesized that the mosquito populations collected and sequenced are probably from the same origin with those from other parts of the world, although there is no evidence of their dispersal routes. This observation is supported by previous studies which suggested that *Culex* is not a monophyletic group (Rozo-Lopez & Mengual, 2015). There were also low genetic variations in the DNA sequences of mosquito species both within and between species. DNA nucleotide base substitutions (transition and transversion) were observed among the mosquito species studied. In all the sequenced *COI* samples, there was 230 variable sites the highest being A → T transversions within the *Culex pipiens* sequences, there were three variable sites which is G ↔ A transition, there were 22 variable sites between the three *Culex antennatus* species which include 14 transversions and 8 transitions. There are also 173 variable sites among all the Culex species sequenced. These results are consistent with previous study of Shouche and Patole (2000) where they also reported a high A-T type transversion in both 16S rRNA and *COI* sequences.

Conclusions

This study concludes that the reliability of the use of ribosomal *IGS* and mitochondrial *COI* gene regions for molecular diversity study and precise identification mosquito species in North-Central Nigeria because they can provide sufficient information on the mosquito species diversity present in this region. Both regions were able to show the evolutionary relationship amongst the mosquito species as well as infer the molecular divergence existing between and among them. Further investigation into the evolutionary relationship and divergence among species complexes with larger sample size is therefore recommended and this could be achieved through DNA barcoding and population genetic structure of mosquito species in North-central Nigeria. We therefore suggest increased researches on applications of genetics and population genomics in mosquito control strategies to effectively wage war against malaria.

Abbreviations

An.: Anopheles; PCR: Polymerase chain reaction; DNA: Deoxyribonucleic acid; sp: Species; FCT: Federal Capital Territory.

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Authors' contributions
OAI conceived and designed this study; ATK-I, HAA and RDS collected and preserved the samples; OAI, TOF, ATK-I and RDS performed the molecular laboratory experiments; OAI, ATK-I, OS and RDS analysed the data; OAI and ATK-I wrote the draft of the manuscript. All authors read and approved the final version of the manuscript.

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Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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