Journal of Experimental Research

MARCH 2023, Vol 11 No 1

Email: editorinchief.erjournal@gmail.com

editorialsecretary.erjournal@gmail.com

MOLECULAR CHARACTERISATION OF MALARIA VECTORS IN IJEBU-NORTH LOCAL GOVERNMENT AREA, SOUTHWESTERN NIGERIA

Sulaimon A. Aina[1,*], Olusegun A. Lawal[1], Olufemi M. Agbolade[1], Titilola F. Salisu[1], Benjamin T. Thomas[2], Ismail B. Onajobi[2], Mistura T. Adeleke[1], Ahmed O. Olasupo [3], Ayoola T. Adeoye[1] and Damilola R. Agarawu[1]

[1]Department of Zoology and Environmental Biology, Faculty of Science, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.
[2]Department of Microbiology, Faculty of Science, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.
[3]Department of Mathematical Sciences, Faculty of Science, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.

***Author for Correspondence: :.** aina.sulaimon@oouagoiwoye.edu.ng

ABSTRACT

Literature has confirmed the life-threatening vectoral nature of female *Anopheles* mosquitoes. Paucity of data on the composition of the species and diversity of *Anopheles* mosquitoes that transmit malaria disease in Ijebu-North Local Government Area (INLGA) of Nigeria necessitates this present study with the main objectives anchored on molecular diversity and entomological indices of malaria vectors. Six communities (Ojowo, Oke-Agbo, Oru- Ijebu, Awa-Ijebu, Oke-Igan and Ibipe) were randomly selected for the study with ten (10) apartments from each community. Mosquito samples were collected using Pyrethrum Spray Catch (PSC) method with modified exit trap on a monthly basis for a period of one year. The specimens were preserved and sorted through morphological identification using reference guides. Mosquitoes were characterised molecularly using Polymerase Chain Reaction- Randomly Amplified Polymorphic DNA (PCR-RAPD) method. Molecularly, four species *An. gambiae s.s.*, *An. funestus s.s.*, *An. arabiensis* and *An. leesoni* were identified and which was better identification than the morphological approach. *An. gambiae s.s.* occurred most 90 (33.83%) followed by *An. funestus s.s.* 65 (24.44%), *An. arabiensis* 58 (21.8%) and *An. leesoni* with 53 (19.92%). In conclusion, this study is able to provide a long needed identification of *Anopheles* species that transmit malaria in Ijebu North which is intended to serve as background information for subsequent studies. However, periodic surveillance must be employed in order to update the malaria vector database, while government at all levels should improve funding for research on malaria vectors because of their persistent resistance to insecticides.

Key words: *Anopheles*, Molecular Characterisation, Ijebu North Local Government, Population dynamics,

INTRODUCTION

Malaria is caused by the malaria parasite *Plasmodium falciparum* and vectored by the mosquito *Anopheles gambiae (*Fontenille and Simard, 2004). Despite 50 years of malaria vector control efforts globally, malaria remains a major public health threat in tropical and subtropical countries (Miller and Greenwood, 2002; Sachs, 2002; Spielman et al., 2002). Malarial incidence dropped 18% and the number of malaria deaths globally dropped from an estimated 839 000 in 2000 to 438 000 in 2015, a decline of 48%. But most cases and deaths in 2015 were estimated to have occurred in the WHO African Region (88%), and followed by the WHO South-East Asia Region (**Gunathilaka** et al.**,** 2015; WHO, 2016). And there has been an increase in the incidence rate as well as death rate in these regions till 2019 (WHO, 2019).

Better management strategies for mosquito-borne diseases have been possible by studies on species composition and density of local mosquito populations (Ashfaq et al., 2014). *An. gambiae* and *An. funestus* complexes (are morphologically the same, but they are genetically and behaviorally different species) have been the dominant *Anopheles* vectors of human malaria in Africa (Okwa, 2019). In Kenya, the common mosquito species transmitting malaria are *An. gambiae s.s*. and *An. funestus s.s*. (anthropophilic) and *An. arabiensis* (zoophilic) (Ondiba et al., 2017). Two other species, *An. pharoensis* and *An. coustani* have been reported in some parts of East Africa as being anthropophylic (Fornadel et al., 2011; Ondiba et al., 2017).

Malaria vector surveillance is one of the cardinal areas of malaria control initiatives genda of the Presidential Malaria Initiative (PMI) in Nigeria. Ijebu-North area covers an expanse of 967km^2 and a human population of more than 284,336 people (in the 2006 CENSUS). With the headquarters in Ijebu-Igbo, it comprises other major towns including Ago-Iwoye, Oru-Ijebu, Awa-Ijebu and Ilaporu which are mostly semi-urban in nature. INLG lies between 6.2°E 7.8° N and 3.0°E 5.0°N, within the rain forest zone. (Ogun State Government, 2016).

Morphological characterisation of mosquito species is obviously limited since it does not consider phenotypic plasticity, genetic variation of individuals or morphological complexity (e.g. cryptic taxa or keys only developed for certain gender or life stage) (Helmersson, 2013). Furthermore, molecular-based methods are generally used in taxonomic studies for species identification of viruses, bacteria and protozoa (Adl et al., 2007).

Molecular characterization involves techniques like DNA extraction, Polymerase Chain Reaction (PCR), Agarose Gel Electrophoresis and Restriction Fragment Length Polymorphism (RFLP) methods and DNA Sequencing while other are the cytogenetic analysis of polytene chromosome (Okorie et al., 2011), usage of internal transcribed spacer 2 (ITS2) or intergeneric spacer (IGS) (Aju-Ameh et al., 2016), analysis of cuticular components (Walton et al.,2007; Aju-Ameh et al., 2016) and sequencing of the cytochrome c oxidase subunit I (COI) gene (known as the barcode segment) (Pfeiler et al., 2013; Weeraratne et al., 2017). Like the barcodes in the grocery store these sequences work for unique identifications of every species (Helmersson, 2013).

Herbert et al. (2003) opined that even though previous barcoding studies have been done on the cytochrome b oxidase gene, but COI is another gene that should be considered. It has areas with relatively conserved sequences and therefore universal primers developed for this gene are very robust. In comparison to other mitochondrial genes, the phylogenetic signal from COI seems to have greater output. Ordinary PCR and Sanger sequencing are now routinely used for DNA barcoding.

Due to several health challenges facing the country, such surveillance plans on vectors of diseases is eminent. Presently, there is paucity of information in literature unveiling the status of malaria parasites and vectors in some areas within Southwest of Nigeria with real time malaria control in which Ijebu-North Local Government is one of those areas. This therefore, necessitated the study of the malaria parasite and vectors in the Local Government.

Centre for Disease Control (2012) and Kiszeweski et al. (2004) reported that for the Southwestern part of Nigeria, the *An.funestus* and *An. gambiae s.s.* are the dominant species when most Northern parts have *An. funestus* and *An. arabiensis* as the dominant siblings, whereas, in some Eastern parts, *An. gambiae s.s.* and *An. funestus* are found to be the most abundantly distributed species.

Cytological methods of identification are not suitable as they are stage-specific, time-consuming and laborious to perform. PCR-based methods of identification are preferable as they are relatively quick, straightforward and reliable. Regions of the ribosomal DNA (rDNA) are often the markers of choice in *Anopheles* for this purpose as there are often fixed differences even between closely related species (Walton et al., 2007). In a survey conducted in rice fields and plantation in Ajana-Liyebi and Ikenne farm settlements using the CDC light trap, out of 47,510 mosquito collected, *Mansonia africana* (63.8%) had the highest population while *Aedes aegypti* had the highest parity rate with 0.81-0.86 (Amusan et al., 2005).

Oyewole et al. (2005) used PCR and multiplex PCR to identify mosquitoes collected from Akaka-Remo, Ilisan-Remo and Ijesa-Isu, all in the Remo Zone of Ogun State, Nigeria. They reported that of all the 1,800 adult females caught, 1,399 (77.7%) were *Anopheles gambiae sensu lato* and 401 (22.3%) were *An. funestus*. In another study carried out in the forest at Alakia, Ibadan, Southwestern Nigeria, Awolola et al. (2007) species identification was done using morphological keys and PCR/ DNA extraction procedures. Oguoma et al. (2010) also reported that out of the 953 mosquitoes collected from three villages of Uratta Owerri North Local Government Area, *Anopheles* mosquitoes, *Anopheles gambiae complex* 536 (56.2%) showed higher preponderance than *An. funestus complex* 306 (32.1%), *An. coustani* 65 (6.8%)

and *An. moucheti* 46 (4.8%).

In view of the established vectorial role of mosquito in the epidemiology of human malaria, this work was embarked upon to assess the genetic diversity of malaria vectors in Ijebu North Local Government Area (INLGA). This is towards updating the malaria vector database in Ogun State, thus providing background information and proffering solutions for sustainable malaria control programs.

MATERIALS AND METHODS

Study Area

This study was conducted in six Health Centre (HC) based communities in Ijebu-North Local Government Area (INLGA) of Ogun State, Nigeria. Most of the residents lack good water drainage system which characterises a semi-urban system. INLGA has a bi-modal rainfall; long rainfalls between March and May and short rains between July and October.

Protocols

Reconnaissance Survey or Pre Survey Protocols

Prior to the commencement of this study, repeated visits were made to the heads of the communities and household heads within the community to get permission to use their apartments for the survey. Malaria drugs were given to households with direction on usage at the end of the study as incentives.

Entomological Sampling/ Specimen Collection

Collection of mosquitoes was done by Pyrethrum Spray Catch (PSC) (Oduola et al., 2012) six (6) communities (Ojowo, Oke-Agbo, Oru-Ijebu, Awa, Ibipe and Oke-Igan), in INLGA. Ten (10) apartments were used in each of the Communities/ Health facilities during the study. This was done between 0600-0730 hours (WHO, 1995; Obembe et al., 2018) once in a month from May, 2017 to April, 2018.

The collected and sorted mosquito samples were kept in separate 1.5ml eppendorf tubes with desiccated silica gel (Gonzalez et al., 2017) overlaid with filter paper (Oduola et al., 2012). Each tube was labeled appropriately with place of collection, time, date of collection and serial number with the name of collector. For molecular procedure, mosquitoes were individually preserved in 80% ethanol, which was rehydrated in water for 1 hour prior to DNA extraction.

Morphological Identification

Samples were morphologically identified with binocular dissecting microscope (XT-3C 20x & 40x dual light illumination turret step stereoscopic microscope/ Student microscope & Biological compound microscope). The morphological identification was done using the keys of Gillies and Coetzee (1987) and Gillet, (1972) in the Department of Zoology and Environmental Biology, Olabisi Onabanjo University, Ago-Iwoye.

Molecular characterisation

Mosquitoes identified as belonging to major vector complex groups such as *An. gambiae s.l.* and *An. funestus s.l.* were separated during the morphological identification process for separate individual PCR (Scott et al., 1993) and sequencing. The DNA extraction and PCR were carried out at the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria, while the DNA sequencing was done at Inqaba Biotech, South Africa.

Genomic DNA was extracted from each mosquito sample using the method of Ander *et al.* (2012). The cytochrome c oxidase II (*COII)* gene of the mitochondrial genome was amplified following Thomas et al. (2017) while extracted DNAs were amplified by PCR-RAPD using universal primers of A-*tLeu 5'-ATGGCAGATT AGTGCAATGG-3'* and reverse primer of *B-tLys 5'-GTTTAAGAGACCAGTACTT G-3'* as described by Liu and Beckenbach (1992); Ihwan et al. (2020) and Ghabeish et al. (2021), at the Molecular Laboratory of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos while the amplified samples were sequenced at the Inqaba Biotech, Pretoria, South Africa..

RESULTS

Morphological assessment of Anopheles

After the morphological identification procedure on the 1,316 anopheline mosquito samples collected, two major complex groups were identified (*Anopheles gambiae s.l.* and *Anopheles funestus s.l.*) (Figures 1–3).

Figure 1:*An. gambiae* (with detached leg) (Mg = x40)

Figure 2:*An. gambiae* (without legs) from the study area (Mg = x40)

Figure 3:*An. funestus* from the study area (Mg = x40)

Molecular Diversity

Figure 4 depicts the amplification of *An. gambiae* DNA which was specifically amplified through *An. gambiae* specific primer. *An. funestus* DNA was also successfully amplified with their specific primers as revealed by their discrete bands (Figure 5). Figure 6 represents the Randomly Amplified Polymorphic DNA (RAPD) typing of the different *Anopheles* mosquito species sampled. After the molecular procedures, four Anopheline species were identified which cut across all the locations where the survey was carried out. The population (n = 266) was highest in *An. gambiae s.s.* 90 (33.83%) followed by *An. funestus s.s.* 65 (24.44%), *An. arabiensis* 58 (21.8%) and *An. leesoni* with 53 (19.92%) (Figure 7). Figure 8 revealed the occurrence of *Anopheles* species in the surveyed communities based on molecular diversity.

In all the locations, Oru (*An. gambiae* and *An. funestus*) and Ibipe (*An. gambiae*) had the highest with 23 from the 266 samples analysed molecularly followed by *An. arabiensis*- 19 in Oke-Igan while the least abundant was *An. funestus* in Awa-Ijebu on the basis of Anopheline species abundance (Figure 8).

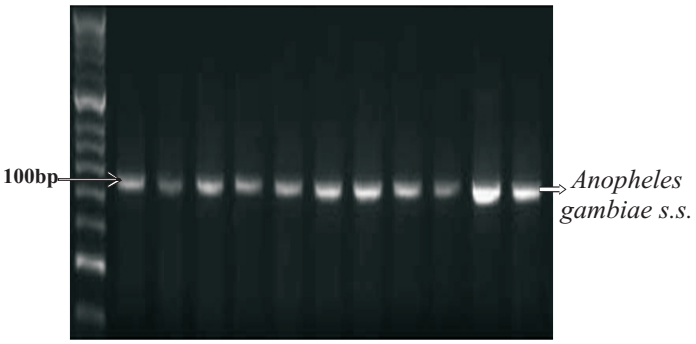
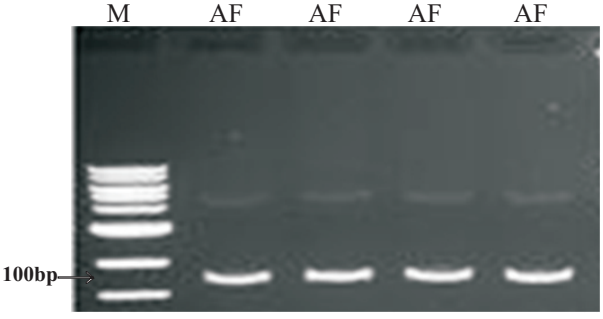
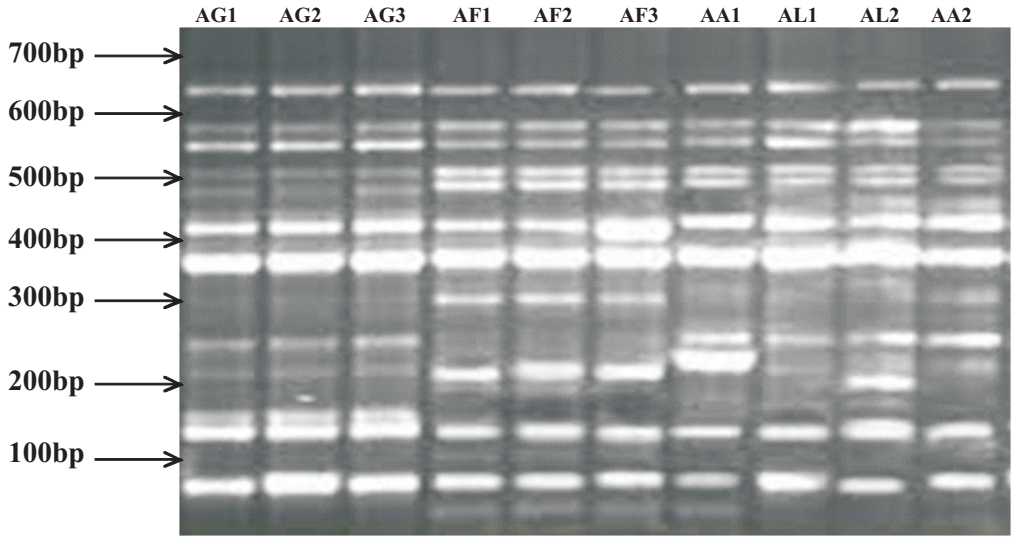


Figure 4: Amplification of DNA of *Anopheles gambiae s.s.* from Ijebu-North, Southwest Nigeria (DNA Marker – 100bp).



M = DNA Ladder **(100bp)**, **AF** = *Anopheles funestus*

Figure 5: Amplification of *Anophelesfunestus s.s.* DNA from Ijebu-North, Southwest, Nigeria



AG = *Anopheles gambiae*, **AF** = *Anopheles funestus*, **AA**= *Anopheles arabiensis*, **AL** = *Anopheles leesoni*

Figure 6: RAPD typing of *Anopheles* mosquito species from Ijebu-North, Southwest Nigeria

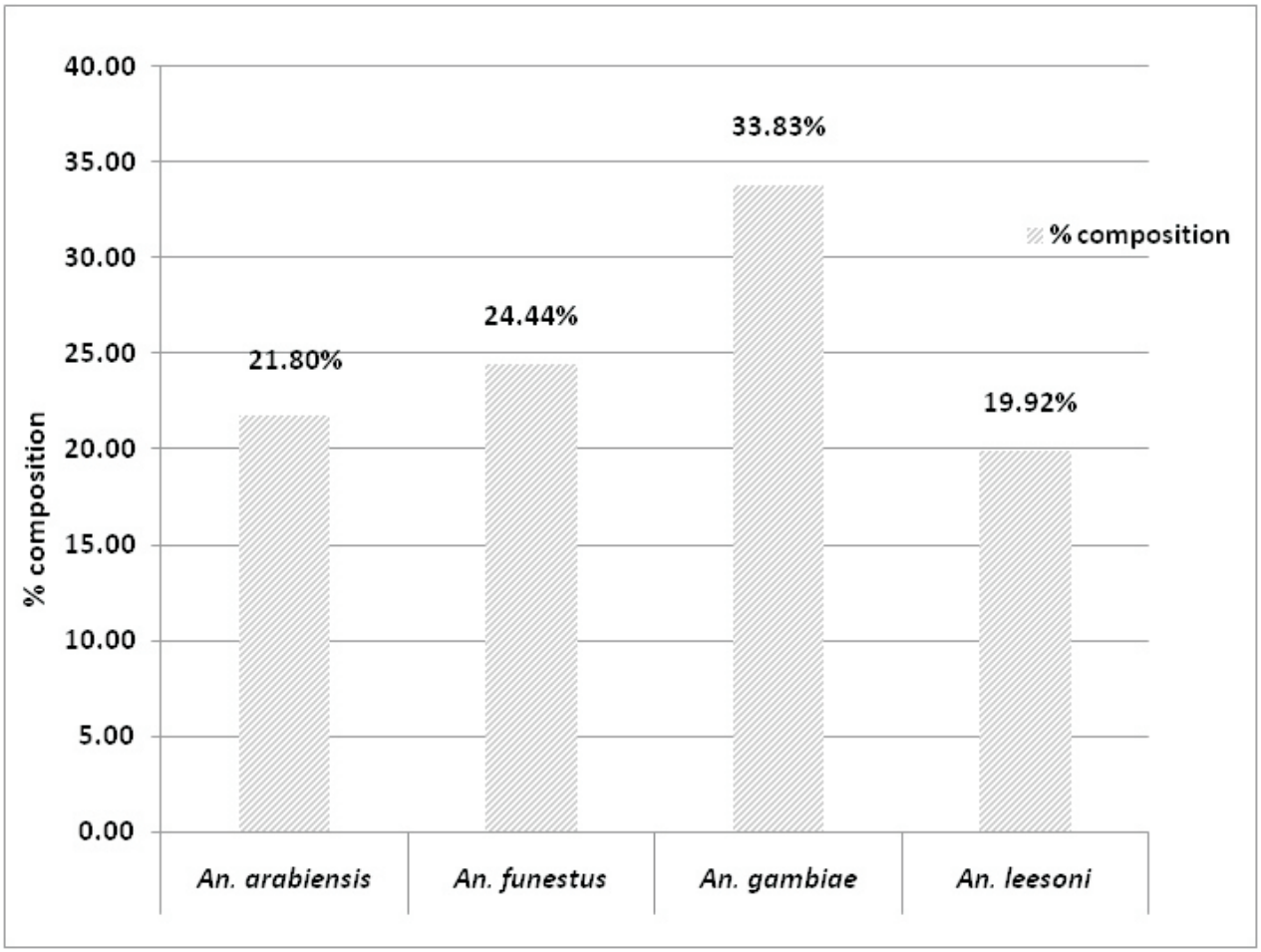


Figure 7: Prevalence of *Anopheles* mosquito based on species

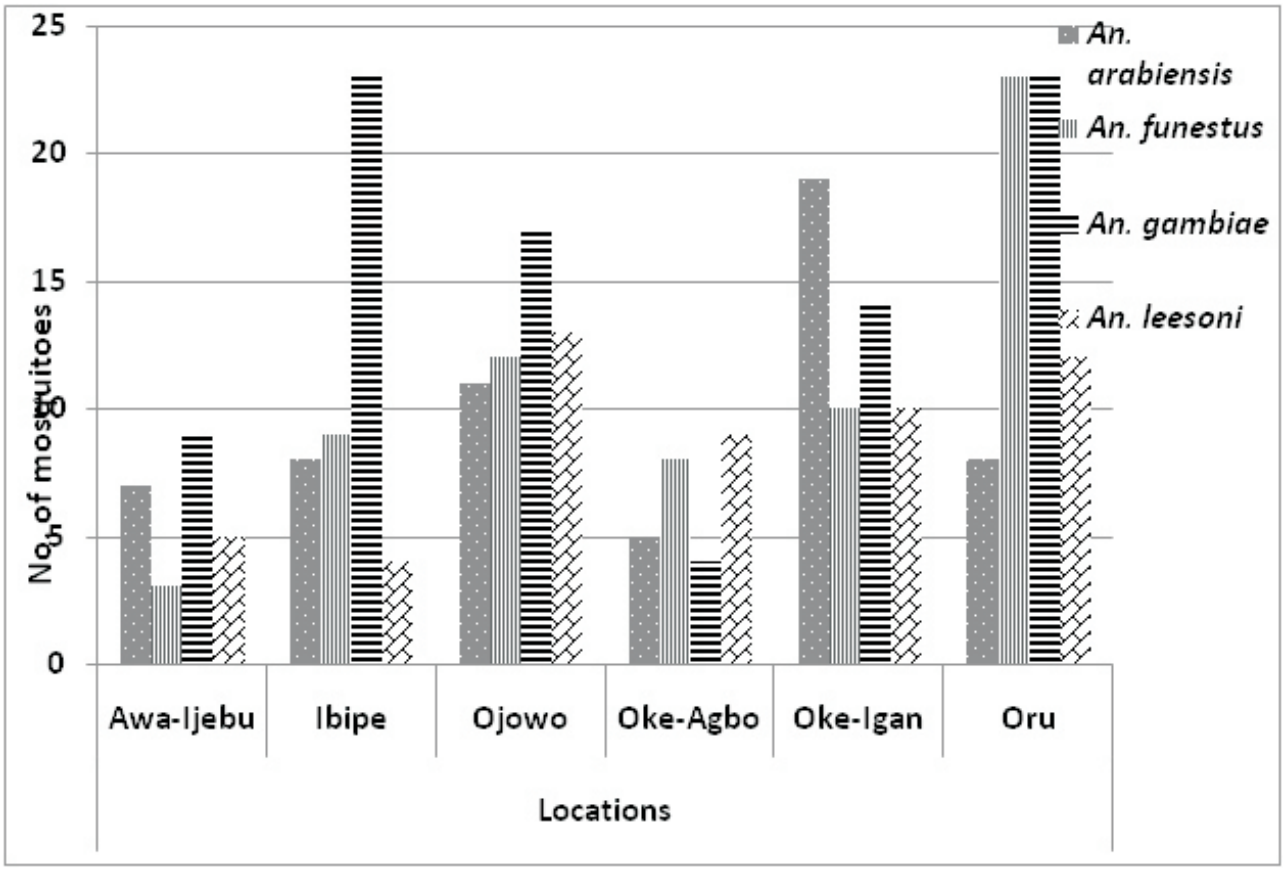


Figure 8: Location-based malaria vector distribution in INLGA with molecular diversity

The Random Amplified Polymorphic DNA (RAPD) strain diversity of the studied mosquitoes in INLG is shown in Table 1. The RAPD analysis of *An. gambiae* (AG) shows similar haplotypes as revealed by their band patterns. All the RAPD bands of AG were similar while *An. funestus* (AF) differs from AG by the presence of RAPD bands 400bp, 560bp and lack of 350bp. *An. leesoni* (AL) on the other hand was discriminated from other species of *Anopheles* at locus 290bp. As for *An. arabiensis* (AA), their point of difference to AG was at 550 and 560bp, while to AF was 300 and 400bp. Some strains of AA also differ at RAPD band 620bp to depict variation in their banding patterns. This observation was further corroborated by the RAPD band frequency (%) in Table 2.

The RAPD markers and the number of bands obtained with each of the markers after appropriate optimization are represented in Table 3. OPM-06, 11, 12, OPM-01, OPM-15 and OPM-19 delineated 18, 20, 25, 28, 27 and 32 bands, respectively while their respective number of polymorphic bands is 10, 10, 11, 12, 08 and 21. The genetic diversity indices for AG connotes disparity in the percentage of polyloci bands with AG having an estimated value of 38.89%, AA 29.17%, AF having 23.61% and AL having the lowest value of 8.33%. The number of alleles per loci was approximately 1.2, 1.3, 1.4 and 1.5 for AG, AF, AA and AL, respectively (Table 4). The total Shannon index of diversity approaches 0.3 for all the Anopheline mosquitoes. The expected Bayesian heterozygosity also varies between 0.21 and 0.24.

Table 1: RAPD scoring of polymorphic bands in the studied *Anopheles* mosquito species

No. bp	AG1	AG2	AG3	AF1	AF2	AF3	AA1	AA2	AL1	AL2
100bp	+	+	+	+	+	+	+	+	+	+
200bp	+	+	+	+	+	+	+	+	+	+
290bp	-	-	-	-	-	-	-	-	-	+
300bp	-	-	-	+	+	+	-	-	-	-
310bp	-	-	-	-	-	-	+	-	-	-
350bp	+	+	+	-	-	-	+	+	+	+
400bp	-	-	-	+	+	+	-	-	-	-
450bp	+	+	+	+	+	+	+	+	+	+
500bp	+	+	+	+	+	+	+	+	+	+
550bp	-	-	-	+	+	+	+	+	+	+
560bp	-	-	-	+	+	+	+	+	+	+
600bp	-	-	-	-	-	-	-	-	-	-
610bp	+	+	+	+	+	+	+	+	+	+
620bp	+	+	+	+	+	+	+	+	+	+
700bp	+	+	+	+	+	+	+	+	+	+

No. bp = Number of base pairs, **AG** = *Anopheles gambiae*, **AF** = *Anopheles funestus*, **AA** = *Anopheles arabiensis*, **AL** = *Anopheles leesoni*

Table 2: Random Amplified Polymorphic DNA band Frequency (%)

BANDS	AG	AF	AA	AL	TOTAL	PROBABILITY
0.10kbp	100	100	100	100	10	1.0
0.20kbp	100	100	100	100	10	1.0
0.29kbp	0	0	0	50	1	0.1
0.30kbp	0	100	0	50	1	0.1
0.31kbp	0	0	50	0	1	0.1
0.35kbp	100	0	100	100	8	0.8
0.40kbp	0	100	0	0	2	0.2
0.45kbp	100	100	100	100	10	1.0
0.50kbp	100	100	100	100	10	1.0
0.55kbp	0	100	100	100	7	0.7
0.56kbp	0	100	100	100	7	0.7
0.60kbp	0	0	0	0	0	0
0.61kbp	100	100	100	100	10	1.0
0.62kbp	100	100	100	100	10	1.0
0.70kbp	100	100	100	100	10	1.0

AG = *Anopheles gambiae*, **AF** = *Anopheles funestus*, **AA** = *Anopheles arabiensis*, **AL** = *Anopheles leesoni*

Table 3: Random Amplified Polymorphic DNA Decamers and their band number

Primer	Sequence	NOBS	NOPBS
OPA – 06	GGTCCCTGAC	18	10
OPA – 11	CAATCGCCGT	20	10
OPA – 12	TCGGCGATAG	25	11
OPD – 01	ACCGCGAAGG	28	12
OPF – 15	CCAGTACTCC	27	08
OPM – 19	CCTTCAGGCA	32	21
Total		**150**	**72**
Mean		**30**	**14.4**

NOBS = Number of Bands Scored, **NOPBS** = Number of Polymorphic Bands Scored

Table 4: Genetic diversity indices for malaria vector in Ijebu-North, Southwest Nigeria

Species	N_P	PPB (%)	N_O	N_E	I	H_E	H_B
AG	28	38.89	1.2012	1.10	0.02	0.014	0.23
AF	17	23.61	1.301	1.08	0.21	0.013	0.21
AA	21	29.17	1.401	1.031	0.03	0.018	0.24
AL	06	8.33	1.513	1.102	0.04	0.013	0.24

N_P = Polymorphic loci, **PPM** = Percentage of polymorphic loci, N_O = Number of alleles per loci, N_E = Effective number of alleles per loci, **I** = Shannon index of diversity, H_E = Nei index of gene diversity, H_B= Expected Bayesian Heterozygocity, **AG** = *Anopheles gambiae*, **AF** = *Anopheles funestus*, **AA** = *Anopheles arabiensis*, **AL** = *Anopheles leesoni*

Anopheles Phylogenetic Tree

The phylogenetic tree of the studied mosquitoes is depicted in Figure 9. The number of clusters obtained was found to be directly proportional to the percentage of tree truncations. At 9.52% level of truncation, the phylogenetic tree was categorised into two major clusters, one was harboring all the *An. gambiae* while the other cluster has the remaining species of Anopheline. At 10.2% level of truncation however, the tree was segregated into three clusters viz; Cluster 1 has all the *An. gambiae*, Cluster 2 has the *An. leesoni* while Cluster 3 contains the remaining Anophelines. Some species of *An. gambiae* were found to have 5% resemblance in their nucleotide sequence with those of *An. arabiensis* and *An. funestus*.

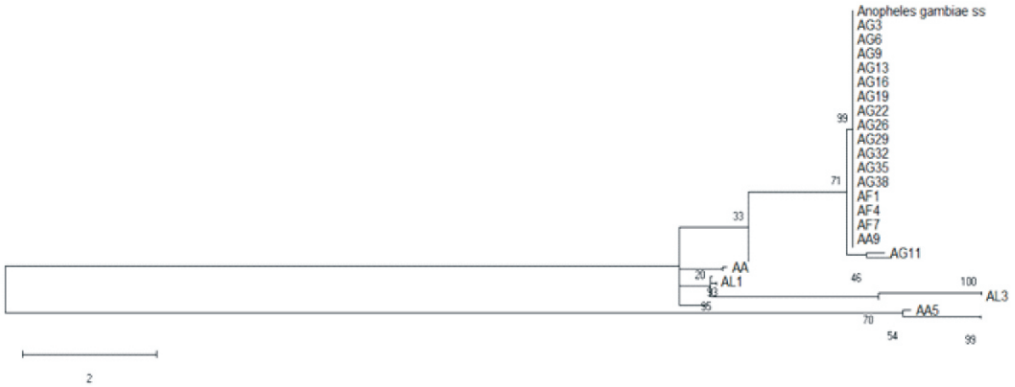


Figure 9: Phylogenetic construction of the sampled Anopheline mosquito species from Ijebu-North, Southwest Nigeria

DISCUSSIONS

Morphological Assessment of Anopheles

Morphologically, two species of malaria vectors (***Anopheles gambiae*** and *Anopheles funestus*) were identified using the morphological method. The two malaria vectors were also reported as the major malaria vector in this part of Nigeria by CDC (2012) and Kiszeweski et al. (2004) in their malaria vector maps. But due to its higher occurrence, *An. gambiae* could be implicated for the huge and persistent transmission of malaria in INLGA of Ogun State. This finding is in line with what was observed in all locations where entomological survey was carried out in Oyo State by Oduola et al. (2012). *An. gambiae* was reported to be most abundant and this could be because the study area falls in the same geo-political and climatic belt as INLGA.

Molecular Diversity

All the *Anopheles* species found in this study exhibited different banding patterns. In *An. gambiae* for instance, the banding pattern of the selected strains were found to be the same for all in terms of their bands to delineate them as the same haplotypes. The same observation also goes for the *An. funestus*. *An. arabiensis* however demonstrated different banding patterns just like *An. leesoni.* Okorie et al. (2011) submitted that the *An. gambiae* complex is the most occurring of all Anophelines in a survey conducted in 2010 across Nigeria. Also that each of *An.gambiae s.s.* and *An.funestus s.s.*is prevalent over the other in at least one locality in each of the five ecological zones of Nigeria and both species coexisted in several localities but in very disproportional numbers. The molecular analysis was found better than the morphological analysis in this study. This is because four (4) *Anopheles* species were identified using the molecular approach namely; *An.gambiae s.s.*, *An.funestus s.s.*, *An.arabiensis* and *An.leesoni*, as opposed to two (2) species detected by morphological approach. In terms of abundance, *An. gambiae* occurred most in the study in line with previously reported works (Ondiba et al., 2017).

An. arabiensis was observed in areas where domestic animals cohabit with the human inhabitant. The presence of domestic animals (goats, domestic fowls, turkeys, dogs and pigs) was observed at most of the collection sites, especially frequently in Oke-Igan (Ago-Iwoye) where domestic fowls and dogs were even noticed in many collection apartments. This is in accordance with the WHO (2013) report which posited that *An. arabiensis* have more presence for animal blood meals, hence tagged "Zoophagic".

Also the more prevalence of *An. gambiae* stressed their inherent anthropophagic preference in taking blood meals. This also reported by Obembe et al. (2018) that the high density of *An. gambiae* could be due to its strong anthropophagic tendencies. The molecular analysis also revealed two major species (*An. gambiae* and *An. arabiensis*) as being dominant which falls in line with the position of CDC, (2012) who reported them as the dominating species in the Southwestern part of Nigeria.

Anopheles **Phylogenetic Tree** Here, two major Clusters were reported which falls in line with the report of Iyiola et al. (2020) who also reported that the phylogenetic tree showed the branching out of the two subfamilies Culicinae and Anophelinae while considering the genetic diversity of mosquitoes in North-Central part of Nigeria. The similarity may be due to the use of the same molecular procedures. There is divergence among the main species as a result of the molecular analysis carried out. *An. arabiensis* formed a distant and separated clade while *An. leesoni* also diverged but related to the *An. gambiae* and *A. funestus* which have co-evolved. This is line with Weeraratne et al. (2017) who reported 12 distinct clades, in which each represents single species.

CONCLUSIONS

This study was able to provide a long needed identification of *Anopheles* species that transmit malaria in INLGA which is intended to serve as background information for subsequent studies. The use of molecular methods in identifying malaria vector is a veritable tool and its equipment be made available in our public institutions for better research output. It also informed Governments at all levels that malaria endemic status as reported is a reality as malarial

mosquitoes in INLGA are almost all infected with the malaria parasite in the study communities. In addition, the use of PSC for mosquito sampling is better than other previously reported methods because it was able to express higher entomological indices.

However, this work was able to give background information on the species of malaria vectors present in the local government area with a view to help in the current and future malaria elimination programs in Ogun State and Nigeria at large. Hence, we recommend that periodic surveillance in order to monitor the molecular diversity and behavioral change in *Anopheles*, unstopped enlightenment on waste disposal, creation of drainages and provision of funding by Government at all levels to make this aspect of malaria elimination program easier.

List of abbreviations

INLGA- Ijebu- North Local Government Area
PCR- Polymerase Chain Reaction
PCR-RAPD- Polymerase Chain Reaction-Randomly Amplified Polymorphic DNA
PMI- Presidential Malaria Initiative
PSC- Pyrethrum Spray Catch
PFLP, Restricted Fragment Length Polymorphism
IGS- Intergeneric Spacer
ITS2- Internal Transcribed Spacer 2
COI- Cytochrome c Oxidase I
CDC, Centre for Disease Control
HC- Health Centre.

DECLARATIONS

Ethics approval and consent to participate

Ethical approvals were given by the Federal Ministry of Health, Abuja (NME/IVM/VS/S.1/01) and Ministry of Health, Abeokuta, Ogun State, (HRS/381/230).

Consent for publication
Not applicable

Availability of Data and Materials

Supplementary information or data can be obtained from the author on request.

Competing interests

The author(s) declare that they have no competing interests.

Funding

Financial supports were offered by the Academic Staff Union of Universities (ASUU), Nigeria for the entomological surveillance and M.O.B.Aina & Sons and Zakat & Sadaqat Foundation (TMC) who both provided financial support for the molecular aspect of the study.

Authors' contributions

SAA, OAL and OMA designed the experiment and preparation of manuscript. The surveillance and morphological identification was done by SAA, ATA and DRA. TFS, BTT, IBO, SAA and MTA were involved in the molecular analysis. OAO TFS and SAA were saddled with the statistical analysis. IBO, MTA and SAA handled the logistics part of the project while OMA, OAL, TFS and SAA participated in the writing of the final versions of the manuscript.

ACKNOWLEDGMENTS

The authors appreciate Prof. O.O. Fafioye, Head, Department of Zoology and Environmental Biology, Olabisi Onabanjo University, Ago-Iwoye for permitting the use of the insectaries in the Animal House. Dr. Dayo Oduola (UNILORIN) for his advice and technical supports.

REFERENCES

Adl SM, Leander BS, Simpson AG, Archibald JM, Anderson OR, Bass D, Bowser SS, Brugerolle G, Farmer MA, Karpov S. (2007). Diversity, nomenclature, and taxonomy of protists. Syst. Biol. **56:**684-689.

Aju-Ameh CO, Awolola SA, Mwansat GS, Mafuyai HB. (2016). Malaria transmission indices of two dominant *Anopheles* species in selected rural and urban communities in Benue state North Central, Nigeria. Intl. J. Mosq. Res. 3(5):31-35.

Amusan AA, Mafiana CF, Idowu AB, Olatunde GO. (2005). Sampling mosquitoes with CDC light traps in rice field and plantation communities in Ogun State, Nigeria. Tanzania. Health Res. Bull. 7(3):111-6.

Ander M, Troell K, Chirico J. (2012). Barcoding of biting midges in the genus Culicoides: a tool for species determination. Med. Vet. EntomoL 27(3):323-331. doi:10.1111/j.1365-2915.2012.01050.x.

Ashfaq M, Hebert PDN, Mirza JH, Khan AM, Zafar Y,

Mirza S. (2014). Analyzing Mosquito (Diptera: Culicidae) diversity in Pakistan by DNA Barcoding. PLoS ONE. 9(5):e97268. doi:10.1371/journal.pone.0097268.

Awolola TS, Oduola AO, Oyewole IO, Obansa JB, Amajoh CN, Koekemoer LL, Coetzee M. (2007). Dynamics of knockdown pyrethroid insecticide resistance alleles in a field population of *Anopheles gambiae s. s.* in Southwestern Nigeria. J Vect Borne Dis. 44: 181-188.

CDC (2012). Global distribution (Robinson projection) of dominant or potentially important malaria vectors. 1pp. www.cdc.gov/malaria/about/biology/mosquito/map.html Accessed on 7 March, 2018.

Fontenille D, Simard F. (2004). Unravelling complexities in human malaria transmission dynamics in Africa through a comprehensive knowledge of vector populations. Comp Immunol Micro Infect Dis. 27357–375. doi: 10.1016/j.cimid.2004.03.005.

Fornadel CM, Norris LC, Franco V, Norris DE. (2011). Unexpected anthropophily in the potential secondary malaria vectors *Anopheles coustani s.l.* and *Anopheles squamosus* in Macha, Zambia. Vect Borne Zoon Dis. 11(8):1173–1179. doi: 10.1089/vbz.2010.0082.

Ghabeish IH, Al-Zyoud FA. Hassawi DS. (2021). RAPD analysis and field screening of bread wheat and barley accessions for resistance to cereal leafminer *Syringopais temperatella*. Jordan J Biol Sci. 14(2):309-316.

Gillet JD. (1972). *Common African Mosquitos and their Medical Importance.* John Swain & Co. Ltd., London. 106pp.

Gillies MT, Coetzee M. (1987). A supplement to the Anophelinae Africa South of the Sahara (Afrotropical region), Johannesburg, South Africa. South Afr Inst Med Res. 55:1-143.

Gonzalez C, Molina AC, Leon C, Salcedo N, Rondon S, Paz A, Atencia MC, Tovar C, Ortiz M. (2017). Entomological characterization of malaria in Northern Colombia through vector and parasite species identification, and analyses of special distribution and infection rates. Mal J, 16:431 DOI 10.1186/s12936-017-2076-5.

Gunathilaka N, Abeyewickreme W, Hapugoda M, Wickremasinghe R. (2015). Species composition and diversity of malaria vector breeding habitats in Trincomalee District of Sri Lanka. BioMed Res Intl, Article ID 823810, 10 pages.

Helmersson E. (2013). Molecular identification of mosquito species: Evaluation of a rapid DNA extraction method together with DNA barcoding as a tool for identification of species. B.Sc. Degree project in Biology, Uppsala Universitet. 21pp.

Herbert PD, Cywinska A, Ball SK. (2003). Biological identifications through DNA barcodes. Proc Roy Soc B: Biol Sci. 270:313-321.

Ihwan, Rusydi, Uslam, Widodo N, Hakim L. (2020). Genetic diversity of *Rhizophora mucronala* on the Western coast of Timor Island. Kuwait J Sci. 47(1):65-71.

Iyiola OA, Shaibu RD, Shittu O, Adelaja O, Kamaldeen-Ibrahim AT, Fadipe TO, Alaba AE, Adejuwon SF, Oyinlola BO. (2020). Genetic Diversity and Molecular Characterization of Mosquitoes (Diptera: Culicidae) in North-Central Nigeria using Ribosomal DNA ITS2 and Mitochondrial 16S-DNA sequences. The Iraqi J Vet Med. 44(2):78–91

Kiszeweski A, Mellinger A, Spielman A, Malaney P, Sachs SE, Sachs J. (2004). A global index representing the stability of malaria transmission. Am J Trop Med Hyg. 70:486-498.

Liu H, Beckenbach AT. (1992). Evolution of the mitochondrial cytochrome oxidase II gene among 10 orders of insects. *Mol Phylogen Evol.* 1(1):41--52.

Miller LH, Greenwood B. (2002). Malaria – a shadow in Africa. *Sci.* 298:121-122.

Obembe A, Popoola KOK, Oduola A, Awolola ST. (2018). Mind the weather: a report on inter-annual variations in entomological data within a rural community under insecticide-treated wall lining installation in Kwara State, Nigeria. Par Vect. 11:497. https://doi.org/10/1186/s13071-018-3078-z.

Oduola AO, Otubanjo OA, Olojede JB, Oyewole IO, Awolola TS. (2012). Malaria transmission risk indices of three *Anopheles* species in selected rural communities in Oyo State South-Western Nigeria. Intl J Trop Med. 7(1):42-48. ISSN: 1816-3319.

Ogun State Government Nigeria (2016). Ogun State profile. 3pp. www.ogunstate.gov.ng Accessed on 23 September, 2013.

Oguoma VM, Nwaorgu OC, Mbanefo EC, Ikpeze OO, Umeh JM, Eneanya CI, Ekwunife CA. (2010). Species composition of *Anopheles* mosquito in three villages of Uratta Owerri North Local Government Area of Imo State Nigeria. Rev Infect. 1(4):192-196. ISSN: 1837-6746.

Okorie PN, Mckenzie FE, Ademowo OG, Bockarie M, Kelly-Hope L. (2011). Nigeria *Anopheles* vector

database: an overview of 100 years' research. PLoS ONE 6(12): e28347. doi:10.1371/journal.pone.0028347.

Okwa OO. (2019). *Nipping the Malaria Vectors in the Bud: Focus on Nigeria*. IntechOpen (online book publisher). DOI: http://dx.doi.org/10.5772/intechopen.83554.

Ondiba IM, Oyieke FA, Ochieng AO, Anyoka DN, Nyanmongo IK, Estambale BBA. (2017). Malaria vector species distribution and seasonal population dynamics across varied ecological zones in Baringo County, Kenya. J Mosq Res. 7(21):174-183 (doi:10.5376/jmr.2017.07.0021).

Oyewole IO, Ibidapo AC, Oduola AO, Obansa JB, Awolola ST. (2005). Molecular identification and population dynamics of the major malaria vectors in a rainforest zone of Nigeria. Biokem. 17(2):171-8.

Pfeiler E, Flores-López CA, Mada-Vélez JG, Escalante-Verdugo J, Markow TA. (2013). Genetic diversity and population genetics of mosquitoes (Diptera: Culicidae: *Culex* spp.) from the Sonoran desert of North America. The Sci W J. ID724609, 11 pages. http://dx.doi.org/10.1155/2013/724609.

Sachs JD. (2002). A new global effect to control malaria. *Sci.* 298:122-124.

Scott JA, Brogdon WG, Collins FH. (1993). Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. Am J Trop Med Hyg. 49:520-529.

Spielman A, Kitron U, Pollack R. (2002). Time limitation and the role of research in the worldwide attempt to eradicate malaria. *J Med Entomol.* 30:6-19.

Thomas BT. Ogunkanmi LA, Iwalokun BA, Agu GC. (2017). Transcriptional factor influence on OTA production and the quelling attribute of sirna on the OTA producing strains of *Aspergillus* section *Nigri*. Afric J Clin Exp Microb. 18(4):210-217.

Walton C, SomboonP, O'Loughlin SM, .ZhangS, Harbach RE, Linton YM, Chen B, Nolan K, Duong S, Fong MY, Vythilingum I, Mohammed ZD, Trung HD, Butlin RK. (2007). Genetic diversity and molecular identification of mosquito species in the *Anopheles maculatus* group using the ITS2 region of rDNA. Infect Gen Evol. 7:93-102.

Weeraratne TC, Surendran SN, Reimer LJ, Wondji CS, Perera MDB, Walton CP, Karunaratne SHP. (2017). Molecular characterization of Anopheline (Diptera: Culicidae) mosquitoes from eight geographical locations of Sri Lanka. Mal J. 16(2):234. doi: 10.1186/s12936-017-1876-y.

WHO (1995). *Vector control for malaria and other mosquito borne diseases*. Geneva, Switzerland: WHO Technical Report series 857, World Health Organization.

WHO (2016). *World Malaria Report 2015*. 32pp.

WHO (2019). *World Malaria Report 2019*. WHO, Geneva. 185pp. Downloaded from www.whoint/malaria.