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Dynamics of *Anopheles gambiae* s.l (Diptera: Culicidae) Sibling Species Composition and Biting Preference in Osun State, Southwestern, Nigeria.

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Abstract

The present study reports the predominant sibling species of An. gambiae s.l in Osun state. Adult mosquitoes were caught quarterly in three Local Governments across the state between 1800hr – 0600hr using the Centre for Disease Control (CDC) light trap and 0600hr – 0700hr for pyrethrum spray catch (PSC) using protocol by the WHO and identified using morphological keys. Molecular analysis for sibling species identification was conducted using polymerase chain reaction (PCR).The CDC light trap had a total of ninety (90) catches while the PSC had a relatively low number (1) of catch. Four (4) mosquito genera were identified: Anopheles, Mansonia, Culex and Aedes. An. gambiae s.l was the predominant mosquito species ($p < 0.05$). The CDC first quarter catch was the highest while the fourth lowest ($p > 0.05$). The outdoor catch was higher than the indoor catch ($p > 0.05$). The biting peak was highest in the first quarter outdoor catch between 02:00-03:00 and 04:00-05:00 am. Molecular result revealed An. coluzii was the predominant sibling species. The present study therefore reports An. coluzii as the current predominant An. gambiae s.l cryptic species in the state. It also shows the exophagic and exophilic biting and resting preference of the species. Therefore, the necessity to adjust

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the current malaria vector control approach method employed for malaria and other mosquito-borne disease (MBD) control and elimination in the state.

Keywords: infectivity, Anopheles, sibling species, dynamics, Osun State, biting preference

INTRODUCTION

Hitherto, vector-borne diseases continue to pose a great public health concern globally due to their implication in the morbidity and mortality particularly in sub-Saharan Africa (Adeleke *et al*., 2018).

Mosquitoes are vectors that constitute serious biting nuisance and transmit most deadly and life-threatening mosquito-borne diseases (MBDs) such as malaria, dengue fever, yellow fever and bancroftian filariasis (Adeleke *et al*., 2013). They are regarded as the most dangerous animals on earth (WHO, 2020). However, female mosquitoes of the *Anopheles gambiae* complex which include *An. gambiae sensu stricto, An. arabiensis, An. funestus, An. rufipes, An. pharoensis, An. wellcomei, An. squamosus, An. coustani, An. maculipalpis, An. nilli,* and *An. pretoriensis* of which two species; *An. gambiae* and *An. funestus* are regarded as the main vectors (Oyewole and Awolola, 2006; Oduola *et al*., 2010; 2012) involved in the transmission of the malaria parasites (*Plasmodium spp*) due to their haematophagous behavior. Similarly, they are also involved in the transmission of arboviral diseases (WHO, 2020).

In 2022, 249 million estimated malaria cases was reported in 85 malaria endemic countries and an increase of 5 million cases compared with 2021(WHO, 2023). The increase in the case numbers over the past 5 years occurred in countries in the WHO African Region. This involves 93.6% of cases and 95.4% of deaths globally; 78.1% of all deaths in this region were among children aged under 5 years in 2022, compared with 90.7% in 2000 (WHO, 2023). *Plasmodium falciparum* is the main species of malaria parasite that is found in Nigeria and responsible for over 80% of total malaria burden while *Wuchereria bancrofti* is responsible for lymphatic filariasis (FMoH, 2013). Nigeria accounted for 26.8% of almost half of the global cases of malaria in 2022 (WHO, 2023). Similarly in 2023, it accounted for 31.1% of half of all malaria deaths globally (WHO, 2023).

The spade in the emergence of new sibling species of *An. gambiae* s.l across the country and their predominance is of paramount public health importance in the control and elimination of malaria and other *Anopheles*-transmitted diseases (ATDs). Furthermore, this undoubtedly could necessitate a drift in the strategies and approach employed in vector control with a view to disease elimination. *An. coluzii*, has been reported to be the predominant sibling species of the complex in Lagos according to a study by Coetzee *et al*., (2013), while Adeleke *et al*., (2018) and Oduola *et al*. (2012) have identified *An. arabiensis* and *An. gambiae* s.s. as the predominant species in Osun and Oyo states respectively.

The effect of climate change have been reported to influence this species predominance transformation and its effect on malaria vector control (Heather and Nicodem, 2024). Oyewole and Awolola (2006) have attributed this to ecological factors. There is presently a paucity of report on the predominant *An. gambiae* s.l across the state despite its endemicity for malaria. Reports on it have been location specific and few. Sequel to this and the continuous change in the environment and climatic indices globally through climate change, there is the crucial need to assessing the *An. gambiae s.l* predominant sibling species in the state with a view to

ascertaining the efficacy of the present malaria and MBDs interventions or the necessity for a change in vector control approach.

MATERIALS AND METHODS

Study Area

The study was conducted in three local government cutting across the three senatorial districts of the state. They are Ido-Osun (N7.779272 and E4.480356), Ife (N7.485694 and E4.556917) and Inisa in Osun West, Osun East and Osun Central senatorial districts respectively.
The state is known majorly for tourism due to the presence of ancient cultural edifices. The major occupation of the inhabitants is agriculture and trading.

Ethical clearance

Ethical clearance was obtained from the Department of Health Planning, Research and Statistics, Ministry of Health, Osogbo, Osun State (OSHREC/PRS/569T/174).

Community Entry and Mobilization

Prior to the commencement of the study, visitation was made to all the study communities to create awareness and enlightenment on the vitality of the study through the community heads and heads of primary health centres. In addition, the purpose of the research was explained to the residents and its benefit to the residents and the state at large.

Adult *Anopheles* Mosquito Collection

Collection of adult *Anopheles* mosquito using Centre for Disease Control (CDC) light trap

Adult *Anopheles* mosquito were collected using procedure by the WHO. Collection was done quarterly (between January 2023 to December, 2023) in each of the three study locations using the CDC light trap approved by the WHO. The adult *Anopheles* were collected between 1800-0600hr in each location for two days with an hourly catch recording. Two CDC light traps were set up in each location with one indoor and the other outdoor for both indoor and outdoor catches respectively. The light traps were placed close to the leg of the occupant of the room in each location while sleeping under an untreated mosquito net. The collected mosquitoes were demobilized in a chloroform container and afterward kept in collecting cups where they were carefully covered using foil to prevent losing them and preserving them till they were transported to the labouratory of the Zoology Department of the Osun State University, Osogbo, Osun State, Nigeria. Each collecting cup was well labelled showing the hour of collection and whether the catch was indoor or outdoor. The cups are properly covered with foil paper and fastened with a rubber band to prevent mosquito loss. The mosquitoes were then transported to the labouratory of the Department of Zoology, Osun State University, Osogbo, Osun State where they were morphologically identified using both the digital and conventional dissecting microscopes using keys by Gillies (1972) and Coetzee (2020). The mosquitoes after identification were preserved in 1.5ml Eppendorf tubes containing silica gel for further molecular analysis.

Collection of adult *Anopheles* mosquitoes using Pyrethrum Spray Catch (PSC)

Ten rooms were selected for PSC in each of the three study areas used for adult mosquito collection across the state. Each room was sprayed using a pyrethrum-based insecticide at 0600hr in the morning at the end of the CDC catch due to the anthropophilic nature of the mosquitoes before they fly out. Prior, to spraying the rooms, a white cloth was spread cutting across the four walls of the room to ensure the easy identification and collection of knocked-

down mosquitoes. After about 5mins, knocked-down mosquitoes are picked into well-labelled petri dishes using forceps. The Petri dishes were properly wrapped with paper tapes to prevent losing the mosquitoes and transported to the same labouratory for analysis.

Molecular Identification of Sibling Species of *An. gambiae s.l*

DNA Extraction

The genomic deoxyribonucleic acid (DNA) was extracted from the mosquito's tissue of individual mosquitoes using genomic DNA purification kit manufactured by the Nigeria Institute of Medical Research (NIMER), Lagos State, (BIOTECH). The genomic deoxyribonucleic acid (DNA) from 50 randomly selected mosquitoes was extracted by crushing the head and thorax of individual mosquitoes placed in 2ml Eppendorf tube with pestle, then homogenized in 500µl lysis buffer. The mixture is vortex and incubated at 56°C for 10min then centrifuged at 10,000 rpm for 1 minute, after spinning, 200µl of absolute ethanol is added to the tube. The mixture was transferred into a spin column and centrifuged at 10,000rpm for 30 sec, discard the flow-through and blot the collection tube on tissue paper. Addition of 500µl of wash buffer 1 to the spin column, then centrifuged at 10,000 rpm for 30 sec following the discard of flow-through and blotting the collection tube on tissue paper. The spin column was centrifuged again at 12,000rpm for 3 minutes to remove all the traces of ethanol, thereafter; the spin column was placed in another microcentrifuge tube. 50µl of elution buffer was added to the Centre of the column then incubated at room temperature for 1 to 2 mins and centrifuged at 10,000rpm for 1 minute to elute the DNA. DNA was stored at -20 °C for PCR amplification.

PCR Amplification

The Protocol provided by Wilkin *et al*. (2006) was used during the amplification of the extracted DNA for Polymerase Chain Reaction. The DNA cocktail used for species identification contain specific species primers for *Anopheles merus* (5-CAACCCACTCCCTTGACGATG -3), *Anopheles gambiae* (5-GCT TAC TGG TTT GGT CGG CATTG-3), *Anopheles arabiensis* (5-GTGTTAAGTGTCCTTCTCCGTC -3), *An. quadriannulatus* (5-GCATGTCCAAGATGGTTCGCTG -3) *Anoheles colluzzi* (M form; 5-TAGCCAGCTCTTGTCCACTAGTTTT-3) and *Anopheles sensu strecto* (S form; 5-CCAGACCAAGATGGTTCGCTG-3). The prepared master mix contained 2.5µl pre-mix, 0.5 µl of IMP-UN, AR-3T, QD-3T GA-3T, ME-3T, IMP-S1 and IMP-M1 specific primers, 5.5µl ddH_2O for both forward and reverse reaction respectively. Prepared Polymerase Chain Reaction (PCR) master mix of 12.5µl was added into each 200µl tube thereby individual extracted DNA template (1µl) was added to each tube. The amplicons of the PCR products undergo initial denaturation at 95°C for 5min (1 cycle), denaturation at 95°C for 30sec, annealing at 59.2°C for 30 sec followed by extension at 72 °C for 30 sec (30 cycles), final extension at 72 °C for 5min (1 cycle) and final hold at 4°C.

Gel electrophoresis

1.5g of agarose gel with 100 ml of Tris-acetate- Ethylene-diamine tetra acetic acid (EDTA) was used in the electrophoresis of the PCR product. The agarose was melted in a microwave for about 2 minutes and allowed to cool satisfactorily. The gel was stained with 5µl Ethidium Bromide for the visualization and detection of amplified DNA fragments, after cooling, the gel was poured into a clean well-casting chamber and an electrophoresis comb was inserted to create wells into which amplicons were loaded. The cast was placed in the electrophoresis tank containing 1X to cover the gel and wells followed by the removal of the comb from the well. The molecular ladder (5µl) was dispensed into the first well followed by 7µl of each

(amplicons) were appropriately loaded and run at 80v with 150 mA for an hour. The gel was viewed and taken under a UV transilluminator for documentation.

Data Analysis

The data obtained were analyzed using analysis of variance (ANOVA) to determine the significance of adult mosquitoes along the locations. A p-value of less than 0.05 ($p < 0.05$) at a 95% confidence interval (CL) would indicate significance in correlation.

RESULTS

Dynamics of adult mosquito species caught by CDC across the study areas

A total of ninety (90) adult mosquitoes were collected across the study areas through the quarterly collection. A noticeable variation in the species composition of the mosquitoes caught was observed. Four genera of mosquitoes were identified: *Anopheles*, *Mansonia*, *Culex* and *Aedes*. Two species (*An. gambiae* and *An. wellcomi*) were identified in the *Anopheles* genus, one species each in the genera *Mansonia* (*Mansonia uniformis*), *Culex* (*Culex quinquefasciatus*) and *Aedes* (*Aedes aegypti*) respectively.

Anopheles gambiae s.l 58 (64.4%) accounted for the highest number of mosquitoes collected followed by *Cu. quinquefasciatus* 34 (26.7%), *Ae. Aegypti* 5 (5.6%), *M. uniformis* 2 (2.2%) and *An. wellcomi* 1 (1.1%). Furthermore, *An. gambiae* predominated all other species in all the study locations except at Inisa 5 (21.8%) where more *Culex spp* (*Cu. quinquefasciatus*) than *Anopheles* was collected. The difference in species composition was found to be significant ($p = 0.03$; $p < 0.05$). However, observation by comparing other species by location did not show any significant difference. In addition, in descending order of abundance, Ido-Osun, Ife and Inisa had 41, 13 and 5 number of *Anopheles spp* (*Anopheles gambiae* and *Anopheles wellcomi*) respectively. Likewise, Ido-Osun 40 (90.9%) recorded the highest number of *An. gambiae* while Inisa 5 (21.8%) had the lowest. *An. wellcomi* had the lowest catch in Ido-Osun, Inisa and Ife with a catch of 1 (2.3%), 0 (0%) and 0 (0%) respectively. Although with no significant difference, a higher number of *Cu. quinquefasciatus* was caught as compared to *M. uniformis* ($p= 0.55$; $p > 0.05$) and *An. wellcomi* ($p = 0.62$; $p > 0.05$) in all the study locations.

Despite the fact that no *Aedes spp* was encountered at Ido- Osun, it nevertheless had the overall highest mosquito species composition across the study areas ($p= 0.012$; $p < 0.05$) (Table 1).

Table 1: Dynamics of the species composition of adult mosquitoes caught by CDC in the study areas

	Ido-Osun (%)	**Inisa (%)**	**Ife (%)**	**Total (%)**
An. gambiae	40 (90.9)	5 (21.8)	13 (56.5)	58 (64.4)
An. wellcomi	1 (2.3)	0 (0)	0 (0)	1 (1.1)
M. uniformis	1 (2.3)	1 (4.3)	0 (0)	2 (2.2)
Cu. quinquefasciatus	2 (4.5)	16 (69.6)	6 (26.1)	24 (26.7)
Ae. Aegyti	0 (0)	1 (4.3)	4 (17.4)	5 (5.6)
Total	44 (48.8)	23 (25.6)	23 (25.6)	**90**

Dynamics of adult female *Anopheles* mosquitoes caught across the study areas

CDC light trap indoor and outdoor catches across the study area

A total of 58 adult female *Anopheles* mosquitoes were caught during the study period. There was an obvious difference in the abundance of mosquitoes caught both indoor and outdoor across the study locations.

In the first quarter (January – March), there was no significant difference in the outdoor catch which was highest at Ido-Osun 26 (78.8%) and the indoor catch was lowest at Inisa 0 (0.0%) ($p= 0.33$; $p > 0.05$). Likewise, the second quarter recorded the highest catch for the outdoor at Ido-Osun 6 (46.2%) and the lowest at Ife 0 (0%) ($p= 0.22$; $p > 0.05$) including indoor catch 5 (38.4%). However, Ife 5 (50%) in the third quarter had the highest outdoor catch while Ido-Osun had the lowest 2 (20%) ($p= 0.18$; $p > 0.05$). Likewise, Ife had the only indoor catch in the third 3 (30%) and fourth 2 (100%) quarters respectively. In addition, Ido-Osun had the overall highest outdoor catch during the study period with a total catch of 34 adult mosquitoes. No catch was recorded outdoor in any of the study areas in the fourth quarter neither for the indoor nor outdoor catches (Table 2).

Furthermore, the first quarter had the highest number of catch for CDC light trap while the fourth quarter had the lowest ($p= 0.44$; $p > 0.05$). The outdoor catch was highest throughout the study period with 31, 8 and 7 catches respectively ($p= 0.25$; $p > 0.05$). Generally, there was no significant difference in the number of mosquitoes collected for the indoor and outdoor catches and across the quarters in the study areas (Table 2).

Table 2: CDC indoor and outdoor female *An. gambiae* complex caught across the study areas

	1st Quarter		2nd Quarter		3rd Quarter		4th Quarter	
	In (%)	**Out (%)**	**In (%)**	**Out (%)**	**In (%)**	**Out (%)**	**In (%)**	**Out (%)**
Ido-Osun	1(3.0)	26(78.8)	5(38.4)	6(46.2)	0(0)	2(20)	0(0)	0(0)
Inisa	0(0)	3(9.1)	0(0)	2(15.4)	0(0)	0(0)	0(0)	0(0)
Ife	1(3.0)	2(6.1)	0(0)	0(0)	3(30)	5(50)	2(100)	0(0)
Total	**2**	**31**	**5**	**8**	**3**	**7**	**2**	**0**

In= Indoor; Out= Outdoor

Percentages (%) were calculated relative to the total values of indoor and outdoor within a quarter

PSC catches of *An. gambiae* s.l across the study area

The number of adult *Anopheles* caught across the study areas by PSC was relatively very low. Catches were recorded only at Inisa during the first 1(100) and second 1(100) quarters respectively while other locations had no recorded catch (Table 3).

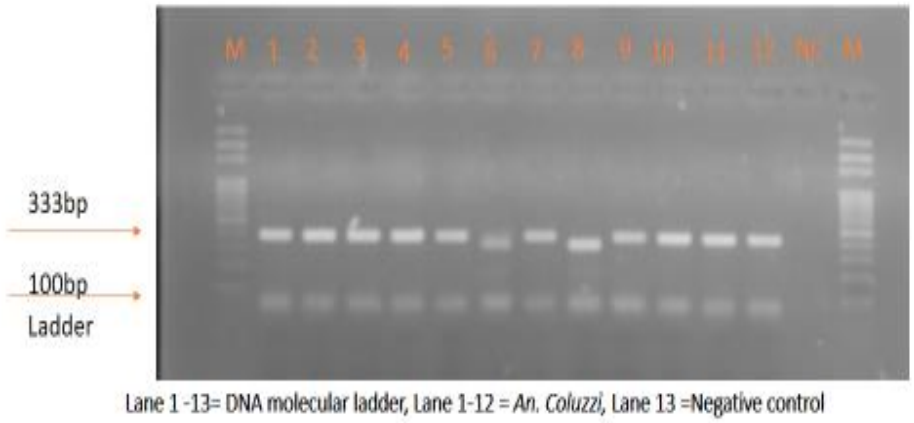
Table 3: PSC catch of adult female *Anopheles* across the study areas

	1st Quarter	2nd Quarter	3rd Quarter	4th Quarter
	Number caught (%)	**Number caught (%)**	**Number caught (%)**	**Number caught (%)**
Ido Osun	0 (0)	0 (0)	0 (0)	0 (0)
Ife	0 (0)	0 (0)	0 (0)	0 (0)
Inisa	1 (100)	1 (100)	0 (0)	0 (0)
Total	1	1	0	0

Percentages (%) were calculated relative to the total values within a column

Molecular Identification of *An. gambiae* s.l sibling species

The female *Anopheles gambiae* complex were subjected to PCR for molecular identification. Only two sibling species of the complex were identified which are *An. coluzzi* and *An. gambiae s.s.* However, no *An. arabiensis* sibling species was identified. In addition, the predominant sibling species identified was *An. coluzzi* with an amplification of 330bp as shown in the gel image below (Figure 1). Furthermore, 99% and 1% of the analyzed mosquito samples were *An. coluzzi* and *An. gambiae s.s* respectively and this spreads across the study areas (Ife, Inisa and Ido-Osun).



Lane 1 -13= DNA molecular ladder, Lane 1-12 = *An. Coluzzi*, Lane 13 =Negative control

Figure 1: Gel electrophoresis plate showing molecular identification of *An. gambiae* s.l sibling species

Biting rhythm of adult female *An. gambiae* s.l

The biting rhythms of the adult female *An. gambiae* complex during the study period indicated that there was a significant variation in their hourly biting behaviour. The overall highest biting peak was observed in the first quarter at 18 (31.0%) between 02:00-03:00 and 9 (15%) between 04:00-05:00 am respectively which occurred for the outdoor catch. However, in the second quarter, the biting peak was at 5 (8.6%) 03:00-04:00 am. In the third quarter, the biting peak between at 01:00-02:00am 3 (5.12%). However, in the first quarter, the biting peak was between 02:00-03:00 am and 20:00-21:00pm for the outdoor and the outdoor catches respectively. The second quarter had both its outdoor and indoor peaks at 03:00-04:00 am. The third peak for the outdoor catch was also at 03:00-04:00 am similar to the second quarter but with an indoor peak at 01:00-02:00 am. No outdoor peak was recorded in the fourth quarter although with an indoor peak at 01:00-02:00 am. The overall biting behavior indicates more exophagic feeding than the endophagic (Figure 2a and b).

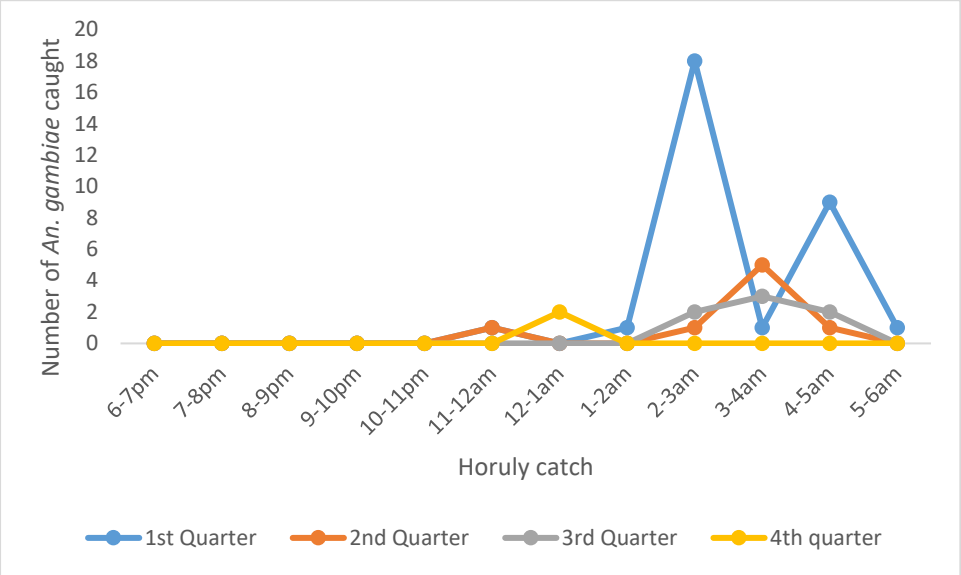


Figure 2a: Outdoor hourly biting rhythm of female *An. gambiae* s.l caught in the study areas

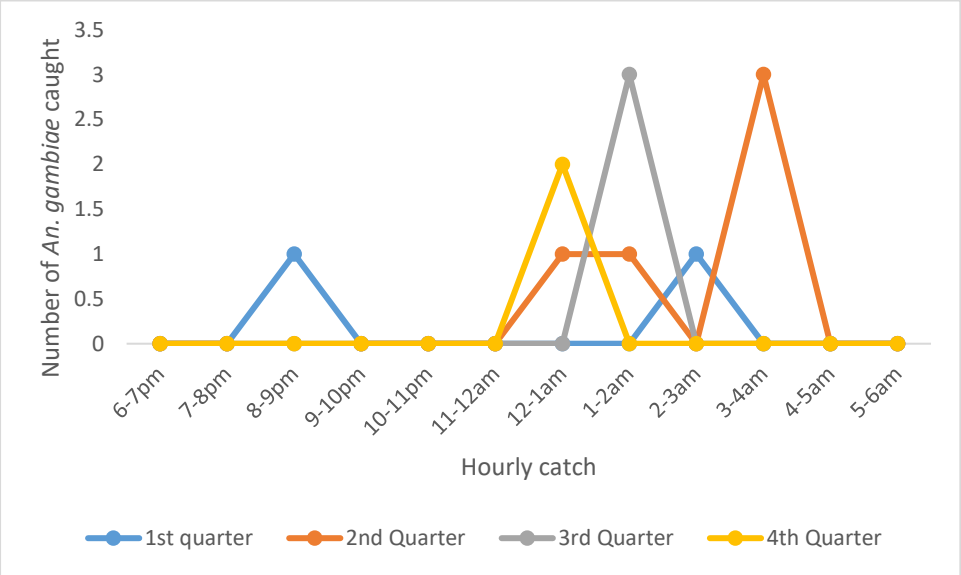


Figure 2b: Indoor hourly biting rhythm of female *An. gambiae* s.l caught in the study areas

DISCUSSION

Mosquito-borne diseases (MBDs) particularly malaria globally continues to pose a great public health threat and concern.

The present study identified four genera of mosquitoes: *Anopheles*, *Mansonia*, Culex and *Aedes* across the study areas. This is consistent with earlier reports by Adeleke *et al*. (2013) in Osun State, Oforka *et al*. (2024) in Lagos state and Onyekachi *et al*. (2017) in Abia state. However, *Mansonia spp* (*Mansonia uniformis*) and *Anopheles wellcomi* have not been earlier reported in Osun State. Although neither of the two species (*M. uniformis* and *An. wellcomi*) are cryptic species of the *An. gambiae s.l* which has seven sibling species. Nevertheless, they also play a key role in the transmission of MBDs in other parts of sub-Saharan Africa such as Tanzania and so on. Furthermore, even though *An. gambiae s.l* and *An. funestus* are the major malaria vector in sub-Saharan Africa, there are also vectors referred to as 'local' vectors which include *An. nili* and seven other secondary or incidental vectors which include *An. wellcomi* and so on

that transmit the disease with a low incidence (Charlwood, 1997). This has been attributed to their low survival rate as suggested by Gillies & De Meillon (1968) for the incidental nature of transmission by many of the secondary vectors. This is because vector longevity is germane for vector competence as only relatively long-lived species are efficient disease vectors (Heather and Nicodem, 2024).

Furthermore, *An. gambiae* was the predominant adult mosquito species caught in the study areas during the study period. This conforms with earlier reports by Oduola *et al*. (2013; 2012) in Osun and Oyo states respectively. The preponderance of this species could be linked to their anthropophilic behavior as suggested by Oduola *et al*. (2013). However, the presence of non-malaria mosquito vector such as *Cu. quinquefasciatus*, *An. wellcomei* and *M. uniformis* portends the extent of residents' risk of exposure to other MBDs and their nuisance. Furthermore, Awolola *et al*. (2002) had earlier reported the predominance of *An. gambiae s.s* and *An. arabiensis* as the major malaria vector in Nigeria. The predominance of *Cu. quinquefasciatus* in Inisa indicates its sympatric association with *An. gambiae* as it is the major vector in the transmission of lymphatic filariasis (LF). Thus, the likelihood of the inhabitant's exposure to LF.

The present study shows that *An. gambiae* s.l bite both indoor (endophagic) and outdoor (exophagic) according to the CDC light trap indoor and outdoor catches across the study areas. However, their peak biting period occurs mainly outdoor than indoor portending their outdoor biting preference. Although this is not statistically significant, however, it is consistent with previous reports by Oduola *et al*. (2021) and Sinka *et al*. (2010). This could be due to the possible use of indoor residual spray (IRS), long lasting insecticide nets (LLINs) and mosquito repellants responsible for preventing and repelling the *Anopheles* mosquitoes from gaining access indoor to feed. Thus, resulting in outdoor feeding (exophagic) before individuals go into their rooms to sleep at night. Therefore, indicating that the indoor use of LLINs is not sufficient in preventing the transmission of the malaria parasite through bite by the mosquito vectors but rather it will only prevent the mosquitoes from biting indoor even though a person could possibly have been infected through mosquito bite outdoor before going to bed. This could be attributed to a behavioural adaptation of the mosquito vectors to counteracting the mosquitocidal effect of the insecticides employed in LLINS and IRS against the vectors. Likewise, similar suggestion was made by Thomson *et al*. (2016b) regarding the outdoor biting preference of *An. arabiensis*, a member of the *An. gambiae* complex, in a study in Ethiopia (Bedasso *et al*., 2022). In addition, Charlwood (1997) reported the transmission of malaria and MBDs in certain localities when people are outdoor, although the most competent vectors are endophagic and malaria transmission majorly occurs indoor. Furthermore, transmission may be dependent on intrinsic factors. However, in contrast, the exophagic biting behavior of the mosquito vectors as reported in the present study contradicts reports by Braack *et al*. (2015) which reported more indoor bite than outdoor. Also, Oduola *et al*. (2013) in a study in Osun State, reported the predominance of indoor resting for *An. gambiae s.s* and therefore suggested the use of LLIN and IRS as an effective control strategy for the mosquito vector. Their report may not be in outright contradiction to result from the present as there could have been a drift in the vector adaptation which makes exophagic as well the anthropophilic nature of the man.

Biting peak was highest in the midnight between 0200-0300hr and 0400-0500hr. This contradicts reports by Ojuka *et al*. (2015) in a study in southwestern, Uganda, where the biting peaks were in the early evening and morning between 1800-2000hr and 0300-0400hr respectively. The variation in biting peaks could be as a result of geographical, environmental

and human anthropogenic activities which could have brought about a drift in the behavioural adaptation of the *Anopheles* vectors to determining the best time for their nocturnal habit of feeding.

The number of *Anopheles gambiae* s.l caught in the wet season was more than that of the dry season ($p > 0.05$). The statistical insignificance of this observation could be due to environmental and climatic factors which could invariably affect the vectorial abundance and density of mosquitoes (Ojianwuna and Enwemiwe, 2022; Hessou-Djossou *et al*., 2022). However, it is in consonance with a previous study by Oforka *et al*. (2024) in Lagos state. This could be attributed to the presence of more rainfall in the wet season than the dry season which invariably leads to the increased availability of breeding habitat for the vectors. However, the abundance or volume of rainfall may not be sufficient in determining vectorial abundance since the physico-chemical parameters of their larva habitat play a significant role in this wise.

Although the number of mosquitoes caught by PSC in the present study was relatively low, the catches occurred as well in the wet season. This could be due to the reported exophagic and endophagic biting and resting preference of the vector which could be borne out of the vector development of behavioural adaptation to evade the mosquitocidal effect of vector control intervention such as LLIN and IRS used indoors.

Molecular identification showed that the preponderant sibling species of the *An. gambiae* s.l caught are *An. coluzzi* (99%) with just 1% of *An. gambiae s.s*. This is consistent with earlier report by Coetzee *et al*. 2013) in Lagos State where *An. coluzzi* was identified as the predominant *An. gambiae* s.l sibling species. However, this contradicts reports by Adeleke *et al*. (2018) in Osun State who reported the preponderance of *An. gambiae s.s.* Therefore, the gradual drift in the replacement of the previously preponderant *An. gambiae s.s* by *An. coluzzi* could be attributed to ecological factors as suggested by Awolola *et al*., (2005); Oyewole and Awolola (2006), and Noutcha and Anumudu, (2009). The effect of climate change cannot be ruled out as well as suggested by Heather and Nicodem (2024) who established the impact of climate change on malaria vector control.

CONCLUSION

This study identifies *An. coluzzi* as the predominant *An. gambiae* s.l sibling species in the state in contrast to *An. gambiae s.s* and *An. arabiensis.* The presence of other mosquito species (*M. uniformis*, *Ae aegypti*, *An. wellcomi* and *Cu. quinquifasciatus*) other than *An. gambiae* s.l predisposes residents to other MBDs such as dengue fever, lymphatic filariasis etc transmitted by other mosquito species aside malaria which is only transmitted by female *Anopheles* mosquitoes.

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