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Taxonomy of *Anopheles* Mosquitoes in Ganawuri Chiefdom, Riyom Local Government Area, Plateau State, Nigeria

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Abstract: Malaria cases occur mostly in poor, rural and indigenous communities in Nigeria. Vector diversity and their spatial distribution are significant indicators to understand malaria transmission dynamics, which can develop effective control mechanisms. The study was developed to determine the composition of *Anopheles* species and their geographic distribution to understand better the malaria transmission dynamics in Ganawuri, Plateau State. Anopheline mosquitoes were captured from selected communities during the rainy and dry season of 2018 using different collection methods described. *Anopheles* species were taxonomically detected using nested PCR through the amplification of mtDNA. A total of 1,820 female adult *Anopheles* mosquitoes were collected, with the most predominant species to be *A. gambiae* s.s 66.48% (n = 1,210), *A. arabiensis* 16.48% (n = 300), *A. melas* 13.46% (n = 245), and *A. merus* 3.57% (n = 65), respectively. Further, the study found that geographical locations significantly affected the distribution of *Anopheles* species ($P < 0.05$) in all the localities that we sampled with Tse. The results of our study have expanded the knowledge on the transmission dynamics associated with anophelines vectors in Ganawuri. Therefore, our study is necessary to expand our knowledge and determine crucial parameters in malaria transmission in Plateau State, which will assist the National Malaria Program in Nigeria towards malaria elimination.

Keywords: Geographical locations, *Anopheles* mosquitoes, Malaria vectors, Nigeria, Plateau State

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Introduction

Anopheles species as vectors of diseases have plagued the world with deadly malaria for decades and centuries now. As one of the most increasing public health, malaria has been a global record of more than 228 million people infected with the disease, while 405,000 deaths were reported by the World Health Organization (WHO, 2019).

Malaria affects the morbidity, mortality, human resources, socio-economic demography of people, and geographical distributions (Padilla *et al.*, 2011). Currently, malaria transmission is found in 21 different countries of the world, with over 132 million people at the risk of infection by this disease (WHO, 2019). The Sub-Saharan Africa

region accounts for the highest malaria cases (90%) and deaths of 91% due to malaria in the world. Of this percentage, Nigeria accounted for the highest malaria in the Sub-Saharan Africa region (27%) (WHO, 2019).

Malaria transmission follows a pattern of the distribution of the anopheline vectors worldwide (Rezende *et al.*, 2009). The genus *Anopheles* is found in almost every part of the world and most of the 465 known *Anopheles* species are not vectors. Still, only 41 species transmit malaria to man (Calderaro *et al.*, 2013). Aside from the *Anopheles funestus*, *Anopheles gambiae* is also one of the dominant and most efficient human malaria vectors in Afrotropical Region (Onyabe, 2001; Sinka *et al.*, 2010; CDC, 2015). This is primarily due to the species' high abundance, longevity, propensity for human feeding, and high vectorial capacity in the Afrotropical Region (Takken, 2003; Autino *et al.*, 2012). Taxonomically, *Anopheles gambiae* complex, referred to as *Anopheles gambiae* sensu lato (s.l.), comprises eight species that are almost indistinguishable morphologically: *A. arabiensis*, *A. gambiae* sensu stricto (s.s.), *A. bwambae*, *A. melas*, *A. merus*, *A. quadriannulatus*, *A. coluzzii*, and *A. amharicus* (Gillies and Meillon, 1964; Gillies and Coetzee, 1987). The variation of malaria vectors and their vectorial capacity distribution may have been affected by environmental, demographic and social factors (Tolulope, 2014; Adepoju and Akpan, 2017; Deme *et al.*, 2017; Torres-Cosme *et al.*, 2021). For example, the annual temperature and precipitation shift may have strongly affected the relative distribution and abundance. *A. arabiensis* and *A. gambiae* in their habitats (Martens *et al.*, 1995; Khasnis and Nettleman, 2005; Pascual *et al.*, 2006; Ostfeld, 2009) and *A. arabiensis* have predominately been reported during the dry season while *A. gambiae* predominately become more abundant during the rainy season (Oyewole *et al.*, 2005). Some studies have shown a fluctuation shift of *A. arabiensis* and *A. gambiae* s.s in ten communities due to temporal fluctuations

(Onyabe, 2001; Awolola *et al.*, 2005; Amadi *et al.*, 2015).

Indeed, some *Anopheles* species have been incriminated as a community. These local or regional vectors are trans-border, and their medical importance at the community level in Nigeria has yet to be determined. Therefore, entomological studies that evaluate the vectorial capacity of other anophelines species are needed to decide on their epidemiological importance. Also of equal importance is the frequent monitoring of entomological parameters of *Anopheles* species such as abundance, composition and their natural habitats. When gathered at local areas, we believe that this information will improve regional and local vector control strategies that can help eliminate malaria in Nigeria. Specifically, in this study, we intend to have entomological research that will help determine the species composition, seasonal distribution, and the detection of natural habitats of *Anopheles* species.

Materials and Methods

Description of the study area:

The study was conducted in Ganawuri Chiefdom, Riyom Local Government Area, Plateau State, Nigeria with an approximate 34 km away from the Plateau State capital city and 150 km away from Nigeria's capital (Abuja).

Study design and site selection:

From each of the six state wards in Ganawuri, we selected the communities that were characterized by being located near a river that ran in both dry and wet seasons and was used as means of water source by other nearby neighbourhoods and surrounded by the ecology of lowlands and grasses. In the communities selected, most households are built with mud blocks and roofed with wooden tin roofs or grasses and might be protected from insect entrances. Therefore, we decided on communities based on the following criteria: (i) its entomological importance with regards to the presence of *Anopheles* vectors,

(ii) the epidemiological importance to the transmission rate of malaria and (iii) the possibility of accessible and safe routes in the community for vector collection.

Collection of adult mosquitoes:

We selected five houses near mosquito breeding sites for the outdoor collection of adult mosquitoes in the chosen communities. From each selected household selected, adult mosquitoes were collected fortnightly in both dry and rainy seasons and from different habitats. We collected mosquitoes in the chosen communities for four consecutive evenings simultaneously once a month, for ten months during September 2018 to August 2019. Mosquito collections were conducted with the assistance of experienced technical personnel trained in capturing malaria mosquito vectors. We reduced bias during collections, rotated the trained field assistants at specific places every day, and followed the technical biosafety recommendations from WHO (WHO, 1993) to minimize the risk of malaria infection in the field team. For indoor mosquito collection, we used Light Traps (LTs) for four consecutive nights simultaneously also. The LTs with octanol nor carbon dioxide (CO₂) was placed at the height of 1.5 m above the ground in the room between the hours of 18:00 and 6:00 (Chamberlain and Sudia, 1967; Service, 1988). We used the traps in houses near mosquito breeding sites with a distance of 25 m.

Taxonomic determination:

We labelled and preserved collected adult mosquitoes using liquid nitrogen (Thermo Scientific™). Each labelled container has information like the locality and date. We then sent samples to the Kunming Institute of Zoology, Chinese Academy of Sciences, China. We identified mosquito species to level using taxonomical keys for adult mosquitoes following Clark-Gil and Darsie (1983) and the Reference Collection at the Entomological Laboratory of the Zoology Department, University of Jos, Nigeria. We used genomic analysis to confirm the identity of

Anopheles species using the mitochondrial COI gene using the PCR polymerase chain reaction (PCR) method of Scott *et al.* (1993). The PCR-amplified products we used were for our mosquito samples excised from agarose gel and purified using the Qiaquick gel extraction kit (Qiagen, Shanghai) following the manufacturer's instructions. DNA sequencing of both strands was carried out using the same primers with an ABI Prism 3500 XL130 sequencer (Applied Biosystems, Foster City, CA, USA). The resulting sequences were edited using Sequencher software. The DNA sequences were compared with *Anopheles* COI sequences available in the GenBank by performing a BLAST search from the National Center for Biotechnology Information Database (<http://www.ncbi.nlm.nih.gov/BLAST/>).

Data analysis:

Mosquito genetic diversity indices and frequency of alleles were calculated per locus for each locality for each species. Mosquito species mean density for *Anopheles* species per state ward was calculated as the geometric mean of the number of mosquitoes captured for the study period. We used separate nested analyses of variance (ANOVA) performed in JMP software, version 3.1 (SAS Institute Inc., Cary, NC), to test for the effect of season or habitats on the diversity and abundance of mosquitoes in the study area.

Results

Anopheles species composition and abundance:

We collected a total of 1,820 female adult mosquitoes, which taxonomically four *Anopheles* species (Fig. 1, Table 1). The most predominant species were *A. gambiae* s.s 66.48% (n = 1,210), *A. arabiensis* 16.48% (n = 300), *A. melas* 13.46% (n = 245), and *A. merus* 3.57% (n = 65), respectively, showing that *Anopheles gambiae* species were significantly dominant in the community ($P < 0.0001$, Table 1).

Effect of Habitat Anopheles species composition and abundance:

Most *Anopheles* species (n = 1,050, 57.69%, $P <$

Table 1: Number of mosquitoes collected based on species

Species	Number collected	Percentage (%)	P value
<i>A. gambiae</i>	1,210	66.48	0.0001
<i>A. arabiensis</i>	300	16.48	0.01
<i>A. melas</i>	245	13.46	0.7
<i>A. merus</i>	65	0.30	0.9
Total	1,820	100.00

Fig. 1: *Anopheles* species in Ganawuri community of Plateau State for a study period of ten months (geometric mean $\pm$ 95% confidence interval).

0.001) were collected during the months that characterized early rains in the localities, in turn a significant number of mosquito species in the months that characterize late rainy season ($n = 770$, 57.69%, $P < 0.05$). This indicates that mosquito breeding registered a lower number during the months in the late rainy season (Fig. 2).

We found that *Anopheles* species were significantly distributed ($P < 0.05$, Table 2) in all the localities that we sampled with Tse, which is the most urban locality in Ganawuri, having the more significant number of *Anopheles* species, and followed by Danto (Fig. 3).

Table 2: Number of mosquitoes collected based on the period

Collection period	Mean Chisq	Df	P-value
Early raining season	1,050	60	0.001
Late raining season	770	30	0.05

Fig. 2: Log number of mosquitoes collected based on the period.

Fig. 3: number of mosquitoes collected in hundred.

Discussion

Anopheles mosquitos' diversity is mostly favored by differences in the local conditions in the various habitats that they occupy to that support the development, dispersion and persistence of their demographical distributions. Although, mosquito population composition and frequency during studies may vary between sampling sites and seasons, therefore, making them to be of great importance for constant monitoring to better understand how different habitat conditions can affect mosquito diversity (Cohuet *et al.*, 2010; Montoya *et al.*, 2017). In this study, we showed that *A. gambiae* s.s was the dominant species in Ganawuri of Plateau State, North-Central Nigeria. We found this species to have the ability to adapt to the diverse habitats, even in habitats which are developed because of human infrastructural activities (Deme *et al.*, 2017; Torres-Cosme *et al.*, 2021). This show that species *A. gambiae* s.s and *A. arabiensis* abundance make the greatest density mosquito species in Ganawuri, Plateau State. Our study recorded a low density of *A. melas* and *A. merus* which we suggest may be due to differences in temperature, human activity, availability of breeding places, rainfall and other environmental factors which may not be favourable to these species (Moreno *et al.*, 2007; Montoya-Lerma *et al.*, 2011).

On our seasonal data, we found that the greatest density of *Anopheles* species was during the rainy season of the year, with species predominant in household that made up Tse and Danto communities of Ganawuri while the dry season of the year recorded the lowest densities across our study area. Our results agree with several other studies that have described patterns of seasonal fluctuation in the composition of *Anopheles* mosquitoes (Voorham, 2002; Girod *et al.*, 2008; Torres-Cosme *et al.*, 2021) and it has shown that an increase in mosquito density may be related to the transition periods between the seasons of a year (Povoa *et al.*, 2001; Achee *et al.*, 2006). However, few studies have revealed no

correlation between the population density of anopheline mosquitoes and precipitation (Montoya *et al.*, 2017; Reis *et al.*, 2018).

Importantly, we showed that the distribution of *A. melas* and *A. merus* species in some areas of communities that may have not been recorded so far (Deme *et al.*, 2017). The new records of these anopheline species further extend the distribution of mosquitoes in Plateau State which may suggest an increase the *Anopheles* mosquito population spread across local communities.

Conclusion

In summary, our study revealed important information about the population dynamics that can influence with anophelines mosquito in Plateau State, North-Central Nigeria. We showed that the most abundant and distributed species were *A. gambiae* s.s, which are mostly found at local communities in Plateau State geographical area (Deme *et al.*, 2017). These findings provide an extended geographical range for *Anopheles* mosquitoes, which some species could act as local malaria vectors.

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