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Species Diversity and Relative Abundance of Anopheline Vectors of Malaria on the Highlands of Mambilla Plateau Northeast, Nigeria

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ABSTRACT

The increase in human population in most African highlands in the recent past has resulted in deforestation and cultivation of natural swamps cconsequently the ecology of African highlands has been changing favoring mosquito survivorship and parasite development. The aim of the study was to determine the effect of altitude on species abundance and diversity of anopheline vectors of malaria along an altitudinal transect on the highlands of Mambilla plateau Nigeria. Adult anopheline vectors were captured by the use of Centre for Disease Control (CDC) modified light traps and Pyrethrum Spray Catches (PSC) while larvae were reared to adulthood. A total of 420 anopheline mosquitoes comprising five species; An. gambiae sl 394(93.81%), An. coustani 17 (4.05%), An. funestus 5(1.19%), An. pharoensis 3(0.71%), and An. rufipes 1(0.24%) were sampled along the altitude locations. A total of 342 (81.42%) adult Anopheles mosquitoes make up the total collection for the study period An. gambiae sl 333(97.36%), was the most abundant of all the adult species collected while An. rufipes 1(0.30%) was the least abundant species. Molecular analysis with PCR showed that Anopheles gambiae ss was the main Anopheles gambiae species in the study area. Peak biting period was between 12am to 2am, temperature and relative humidity had no significant effect on mosquito abundance. There was no significant relationship between altitude and mosquito abundance and species diversity ($P > 0.05$). This study has provided baseline data on the species and diversity of anopheline species on the Mambilla plateau.

Keywords: *Anophelines, Mambilla Plateau, Abundance, Diversity, PCR*

INTRODUCTION

Malaria is caused by four species of protozoan parasites *Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax* and *Plasmodium ovale.* The disease is transmitted by the bite of about 30-40 species of female *Anopheles* mosquitoes of about 515 known species [1]. The World Health Organization policy on Roll Back Malaria program (RBM), placed significant importance on vector control thereby the signing of the Declaration and Plan of Action to reduce malaria burden by the year 2010 by African countries [2]. Reduction was to be achieved through, access to the most desirable measures of protection both at personal and community levels and, population most at risk, children under five years and pregnant women. Countries were to constitute epidemic readiness and warning signals response that are efficient and able to detect and manage promptly any outbreak [2]. Highland regions of Africa are malaria hypoendemic due to climatic factors [3]. Hypoendemicity refers to unstable endemic malaria transmission which is estimated at less than 10% [4]. Topography of highland areas comprise of mountains, valleys and high plateaus with altitudes above 1500m above sea level the climate is cool and temperature inversely related to altitude [5]. Due to variations in highland temperatures which hitherto are low, malaria transmission is unstable leading to an

increase in the number of malaria epidemics in the past decades [6], [7].The increase in human population in most African highlands in the recent past has resulted in deforestation and cultivation of natural swamps [8]. Consequently, the ecology of African highlands has been changing favoring mosquito survivorship and parasite development. Abundance of mosquitoes in the highlands is seen to have resulted from this change in land use [9], [5].

Beside the volatile epidemics, there is an increase of endemic malaria transmission in the African highland regions [10]. Control strategies in highland areas should be much more based on prevention of epidemics through early detection. The challenge however, is insufficient surveillance and response systems to monitor malaria transmission dynamics to guide the elimination process. Data on various entomological parameters of anopheline vectors in high risk regions would provide important information on malaria transmission intensity. These data can aid in scaling up control and assess effectiveness of control measures as well as predict malaria epidemics in the highland regions.

MATERIALS AND METHODS

Study Area

Mambilla plateau is located at longitude 6.8212° N, 11.5345° E and latitude 7.3523° N, 10.7723° E in Taraba state North-Eastern Nigeria. Mambilla plateau has an area of about 3765sq km while the adjoining lowland covers about 1,250sqkm. The topography of Mambilla plateau comprises undulating lowland, low hills and irregular plains, ridges, hills, and escarpment. Climate is semi-temperate with mean annual temperature of 16^0C. Mambilla plateau has an average altitude of 1600m above sea level. Mean annual rainfall is 1800mm; the rainy season extends from late March to October while a short dry season occurs between November and early march. Mambilla plateau has a population of 224,357 people [11].

Study Design

The study was conducted from December 2016 to March 2017 in five communities selected along an altitudinal transect to collect entomological data. The rationale for selection of the communities was based on entomological grounds namely, areas prone to malaria epidemics access road throughout the year and altitude. The communities are Mayo-Selbe (484m), Kakara (1496m), Gembu (1584m), Yelwa (1674) and Nguroje (1885m) above sea level.

Ethical Clearance

Ethical clearance was obtained from Taraba State Ministry of Health and consent of Communities leaders was sought to carry out the research in their various communities.

Field Entomological Survey

Field entomological surveys were carried out in all the five communities, mosquito collections were conducted once every month in each of the study sites from December 2016 to March 2017. Anopheline vectors were captured by two methods the use of Centre for Disease Control (CDC) modified light traps and Pyrethrum Spray Catches (PSC). For the CDC collection, indoor traps were suspended from the ceiling at the foot end of the bed at approximately 1.5meters above the ground level in an occupied room from 6pm to 6am. The outdoor traps were hung on a post around the same houses. After every one hour traps were inspected and mosquitoes were aspirated into clearly labeled paper cups and kept in the cool box for transportation to the laboratory for further processing. PSC were carried out from 6am to 9am in at least 20 rooms in each of the communities by spreading white sheets on the entire floor and over the furniture that could not be moved after which the rooms were sprayed with pyrethrum. The rooms were closed for about 10 to 15 minutes for mosquitoes to be knocked down. The knocked down mosquitoes were collected from the white sheets with forceps into petri dishes. All *Anopheles* mosquitoes collected were then taken to the laboratory and identified morphologically using keys [12], these were sorted out according to species, site, methods, and time of collection, and stored individually in ependorf tubes and silica gel. Anopheles mosquitoes were further identified by molecular means using PCR. Female mosquitoes were classified according to their repletion status into unfed, fed, half-gravid and gravid specimens [13]. Elevations of houses from which both CDC-light trap and PSC samplings were made were recorded.

Data Analysis

$$RD = \frac{NA}{N} \times 100 \quad \ldots\ldots \quad (1)$$

Where RD = relative density of species
NA = number of all specimens of each species collected at each altitude
N = the number of specimens of all species collected at each altitude.

$$H' = -\sum_i \frac{ni}{n} \ln \frac{ni}{n}, \quad E = \frac{H}{H_{max}} \quad \ldots\ldots. \quad (2)$$

Where
H′ = Shannon diversity index
ni= number of species i
n = total number of samples
E = evenness
H_{max} = Maximum Diversity possible

Statistical Analysis

Pearson correlation coefficients were computed on the *Anopheles* mosquito species captured using SPSS 20v to study the correlations between mosquito abundance and altitude.

RESULTS

A total of 420 anopheline mosquitoes comprising five species; *An. gambiae sl*, *An. coustani*, *An. pharoensis*, *An. funestus* and *An. rufipes* were sampled along the altitude locations. *An. gambiae sl* 394(93.81%), was the most abundant of all the species collected followed by *An. coustani* 17 (4.05%), *An. funestus* 5(1.19%), *An. pharoensis* 3(0.71%), and *An. rufipes* 1(0.24%) was the least abundant species. The relative abundance and species of female *Anopheles* mosquitoes along altitude locations are represented in Table 1. Adult *Anopheles* mosquitoes make up 81.42% of the total collection for the study period along the altitude locations. A total of 342adult of which *An. gambiae sl* 333(97.36%), *An. coustani*, 5(1.46%), *An. pharoensis* 2(0.58%), *An. funestus* 1(0.30%) and *An.rufipes*1(0.30%).*An. gambiae sl* was the most abundant of all the adult species collected followed by *An. coustani*, *An. funestus*, *An. pharoensis* and *An. rufipes* was the least abundant species. There was significant difference between number and species of anophelines mosquitoes collected ($P < 0.05$).

Overall greatest species diversity three out of five species were recorded in Yelwa and Mayo-selbe next diversity two species out of five were recorded in Nguroje. Gembu and Kakara recorded the least species diversity (only one species (*An. gambiae*) out of the five anopheline species were collected.

Table1. *Species composition and relative abundance of anopheline mosquitoes on the highlands of Mambilla plateau*

Species Composition	Number sampled	Percentage (%)
Anopheles gambiae sl	394	93.81
Anopheles coustani	17	4.05
Anopheles funestus	5	1.19
Anopheles pharoensis	3	0.71
Anopheles rufipes	1	0.24
Total	420	100

Shannon wiener diversity index of 16.38 and evenness of 10.17 were recorded across the altitudinal locations. Highest relative density was observed in *An. gambiae* (97.37%) followed by *An. coustani* (1.46%) then *An. funestus* (0.59%). *An. pharoensis* and *An. rufipes* had the least relative densities of 0.29% each. There was significant difference between number and species of anophelines mosquitoes collected and altitude ($p<0.05$). Table 2 represents the diversity and relative density of *Anopheles* vectors of malaria along altitudinal locations. Table 3 shows hourly anophelines collection. Peak night biting period along the altitude was between 12-2am. Highest collection 0.18±0.38 and 0.28±0.51 occurred between this period and the least mosquito collections 0.08±0.27 was between 4-6am. There was no significant difference between time of collection and number of mosquitoes collected. There was significant difference between number of hourly night mosquitoes collections by CDC (6pm-6am) and the number collected by PSC (6am-9am) 13.85±36.60. There was a slight increase in indoor temperature and relative humidity over outdoor however there was no significant difference between them.

Polymerase Chain Reaction

Application of species-specific PCR analysis confirmed that *An. gambiae ss* is the only member of the *An. gambiae* complex in all the altitudinal locations. Out of the 300 *Anopheles* species selected [14], 197 (65.7%) were identified as *An. gambiae ss*. The remaining 103(34.3%) could not be identified. This could have resulted from incorrect morphological identification, DNA degradation due to bad preservation or human error. (Figure1).

Table2. *Relative density, species diversity and relative abundance of adult female anophelines across altitudinal locations*

	SPECIES Frequency (%)					
Location	***An. gambiae***	***An. coustani***	***An. funestus***	***An. pharoensis***	***An. rufipes***	**Mean+ SD**
Nguroje	2	0	0	0	1	3
Yelwa	13	2	0	1	0	16
Gembu	58	0	0	0	0	58
Kakara	1	0	0	0	0	1
Mayo-selbe	259	3	2	0	0	264
N	333	5	2	1	1	342
RD	97.37	1.46	0.59	0.29	0.29	100.00
H'	-0.029	-0.063	10.27	3.12	3.12	16.38
E	10.17					

RD = Relative density, N = Number of samples, H' = Shannon weiner diversity index, E = evenness

Table3. *Number of adult mosquitoes caught at different time interval*

Time of collection	Adult mosquito caught	Temperature outdoor	Temperature indoor	Relative humidity indoor	Relative humidity outdoor
6-8pm	0.08 ± 0.27^{a}	23.74 ± 2.98^{f}	23.98 ± 3.09^{f}	36.32 ± 10.30^{b}	34.17 ± 10.16^{b}
8-10pm	0.10 ± 0.38^{a}	21.83 ± 2.55^{e}	22.37 ± 2.53^{e}	40.71 ± 11.69^{bc}	38.46 ± 12.33^{bc}
10-12pm	0.18 ± 0.38^{a}	20.69 ± 2.62^{de}	21.38 ± 2.56^{de}	42.26 ± 12.62^{bc}	40.30 ± 13.23^{bc}
12-2am	0.28 ± 0.51^{a}	19.54 ± 2.62^{cd}	20.33 ± 2.45^{cd}	43.75 ± 12.76^{c}	41.93 ± 13.84^{c}
2.4am	0.13 ± 0.33^{a}	18.60 ± 2.54^{bc}	19.42 ± 2.37^{bc}	44.17 ± 13.50^{c}	43.15 ± 15.18^{c}
4-6am	0.08 ± 0.27^{a}	17.79 ± 2.19^{b}	18.78 ± 2.06^{b}	45.88 ± 14.53^{c}	44.60 ± 15.66^{c}
6-9am	13.85 ± 36.60^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Total	1.19±10.57	18.80±6.27	19.42±6.34	38.94±16.73	37.32±17.11

Values represent Mean ± SD of N=40 and r=5. Values in the same column with different superscript across the row differs significantly p<0.05).

DISCUSSION

All the anopheline species reported in the current study have been reported in other parts of Nigeria [15] and Africa in general. This confirms the wide range of geographic distribution of the anophelines. *An. gambiae sl* was the most abundant of all the anophelines species encountered it was recorded in all the five altitude locations. Several studies have reported similar high abundance of *An. gambiae sl* from different parts of Nigeria [16]; [15]; [17]; [18], it is among the most often reported malaria vector species in the country [15]. *An gambiae* and *An. funestus* were two principal anopheline species identified in the current study this agrees with [16] who recorded these two species out of the three *Anopheles* species recovered in Nassarawa state. *An. gambiae* and *An. funestus* complexes comprised 14.4% of the total species recorded in Benin City Nigeria [18], 65.2% and 17.3% respectively of most commonly reported malaria vectors in Nigeria [15]. Earlier study in other African highlands had reported these two species as the predominant anophelines [19]. This has serious health implications as the two species have been identified as important malaria and lymphatic filariasis vectors in Nigeria [18] particularly so that Nigeria is one of the countries with high malaria prevalence in sub-Saharan Africa [20]; [1].

Several factors have enhanced the vector efficiency of *An. gambiae* in sub-Saharan Africa among which are abundance and close association with humans, its anthropophilic behaviour which increases its vector-human contact [21] and the chance to transmit disease. This calls for concerted effort for the control of these species in order to achieve maximum success in the fight against malaria in Africa and Nigeria as a mechanism to prevent future epidemics of malaria in the highland regions of the country, Mambilla Plateau in particular.

An. coustani recorded the second highest abundance (4.05%) of anophelines along the altitudinal locations. The species is fairly abundant with a wide geographical distribution in many parts of Africa including; Nigeria [15]; Zambia [22], South Africa [7] Kenya and Tanzania [23]; [24] among others. The high demonstration of anthropophilic tendencies is suggestive of its potential as a secondary malaria vector in Africa [22] and although it has

not been implicated with malaria transmission in Nigeria, its presence in the study area is of epidemiological concern. This species may have or develop the potential to be a vector in the future and may play a significant role in malaria transmission in the country.

An. rufipes and *An. pharoensis* had the least abundances of all the anopheline species in the study area this is in conformity with [7] who recorded least abundance of *An. rufipes* 0.1% and confined to only one location of the five locations sampled. Contrary to the present result, *An. pharoensis* and *An. gambiae* jointly accounted for 96.7% of mosquitoes sampled in Baringo in Kenya [25]. *An. rufipes* and *An. pharoensis* have not been incriminated in malaria transmission in the study area and although both species are zoophilic and exophilic they are potential secondary vectors [26].

From our result, a slight change in temperature and relative humidity was observed between hourly anophelines collection however, these did not have any significant effect on the number of mosquito collection.

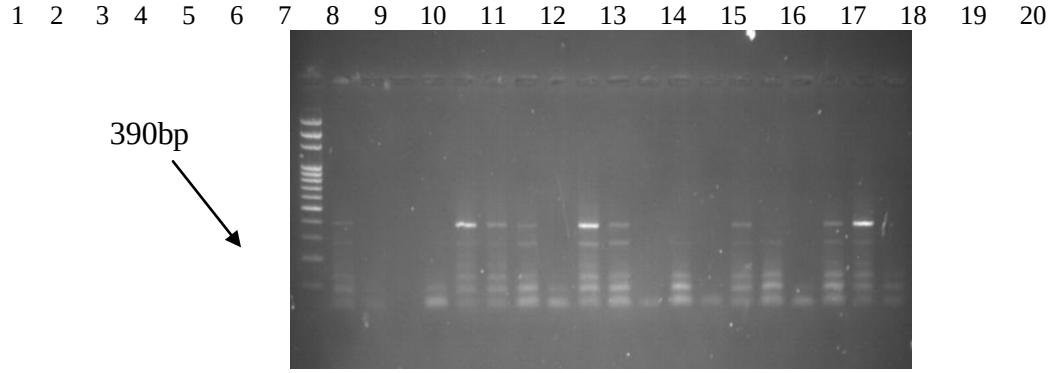

Figure1. *Amplified fragment using the species-specific assay for the identification of member of the Anopheles gambiae complex Lane 1 lader (molecular marker), lane 2 negative control: lane 3 positive control Anopheles gambiaes.s (390bp).*

CONCLUSION

Result of study revealed the abundance of anopheline mosquito species in the study area. Population of *An. gambiae sl* was the most abundant of the five species of anopheline mosquitoes and *An.gambiae ss* as the main anopheline vector of malaria on the highlands of Mambilla Plateau Nigeria. Anophelines mosquito collections were made in all the altitude locations although with varying abundance. With the exception of Kakara (1,496m), mosquito abundance declined either slightly or significantly with increasing altitude.

This study has provided information about diversity of *Anopheles* species on the highlands of Mambilla plateau. This would be helpful for the sustainable management of vector mosquitoes and to take precautionary measures against malaria epidemic. There was no significant relationship between altitude and mosquito abundance and species diversity. This study has provided useful data to elucidate malaria transmission intensity and epidemic on the Mambilla plateau.

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