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Molecular characterization and species composition of anopheline vectors of malaria along an altitudinal gradient on the highlands of Mambilla Plateau Northeast, Nigeria

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Abstract

Diversity of species arises majorly from speciation and this has serious epidemiological implications to disease transmission. The study aimed to determine the species of anopheline vectors of malaria present in five locations along with different altitudes on the highlands of Mambilla Plateau Nigeria. These are; Nguroje (1,885 m), Yelwa (1,674 m), Gembu (1,584 m), Kakara (1,496 m) and Mayo-selbe (484 m) above sea level. Samples were collected by Center for Disease Control (CDC) light trap, Pyrethrum Spray Catches (PSC) and others were reared from larvae. A total of 878 female anopheline mosquitoes comprising of five species namely; *Anopheles gambiae sl* 757(86.22%), *Anopheles coustani* 73(8.31%), *Anopheles funestus* 29(3.30%), *Anopheles pharoensis* 18(2.05%) and *Anopheles rufipes* 1(0.11%). Mayo-selbe had the highest species abundance of 572 but lowest species diversity index of 0.24 *An. gambiae* dominated over other anopheline species 0.76. Yelwa had the least abundance but very high diversity index of 0.81 dominance was 0.41. Out of the 757 *An. gambiae* tested by Polymerase Chain Reaction (PCR), 712(94.06%) were *An. gambiae s.s.*, 192(25.37%) were M form and 520(68.69%) were S form. There was a very strong positive correlation between the M and S forms. As the M forms increase along the locations so also the S forms, $r^2 = 0.94204$.

Keywords: *Molecular, Anopheles gambiae, PCR, M and S form, Mambilla Plateau*

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1. Introduction

About 30-40 species of *Anopheles* mosquitoes are vectors in nature. Most members of the *An. gambiae* are responsible for transmitting *Plasmodium falciparum,* the parasite that causes the most severe form of malaria in sub-Saharan Africa (WHO, 2017). The *An. gambiae* is a species complex that contains seven sibling species that are morphologically identical and indistinguishable. These include; *An. arabiensis*, *An. bwambae*, *An. gambiae sensu stricto*, *An. melas*, *An. merus*, *An. quadrinnulatus A*, and *An. quadrinnulatus B*. The *An. gambiae s.s.* species is

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now undergoing speciation. Five chromosomal forms are now known to include; the Bamako, Bissau, Forest, Mopti and the Savannah as well as two molecular forms these are the M and S forms (Sinka, 2013).

These sibling species exhibit several behavioral differences which enhance their vector efficiency these include their geographical distribution, biting and resting preferences. Other differences exhibited by the sibling species are, resistance to insecticides and host preference (Ebenezer *et al*., 2014; and Wiebe *et al*., 2017). For example, *An. gambiae s.s.*, *An. arabiensis*, *An. quadriannulatus A* and *B* are known to occur in freshwater, while *An. bwambae*, *An. merus* and *An. melas* are found in brackish water (Lehmann and Diabate, 2008). The molecular forms are found in sympatry however, the M form predominates in arid, regions and mostly in irrigated areas or rice fields in West Africa. The S form is dominant in humid, forest habitats across West and East Africa (Lehmann and Diabate, 2008; and Sinka, 2013). Species diversity arises majorly from speciation and this has serious epidemiological implications to disease transmission. New and more efficient vectors might arise and this could further complicate malaria transmission intensity. The malaria geographical distribution and transmission seasons could be extended as a result of these new species. Therefore, the need to determine the anopheline vectors present on the Mambilla Plateau, a highland area prone to malaria epidemics and to characterize the molecular forms of these vectors. This could help to put in place control mechanisms to check the outbreak of malaria infection in the future.

2. Materials and methods

2.1. 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. It has an area of about 3765 sq km while the adjoining lowland covers about 1,250 sq km. It has boundaries with Gashaka Local Government Area in the north-east, Kurmi Local Government Area in the north-west and Republic of Cameroon by an international boundary in the south. It is located in a savannah landscape with a peculiar topography and climate. 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 °C and rainfall of 1800 mm, the rainy season extends from early April to October while the dry season occurs from November to March. Mambilla Plateau has an average altitude of 1600 m above sea level with a population of 224,357 people (NBS, 2012). The people engage in agriculture and stock herding. Crops grown on the Mambilla Plateau include; maize, beans, cocoyam, sweet potatoes and Irish potatoes. Tea, coffee, cocoa and ginger, apple avocadoes and pears are also grown among others.

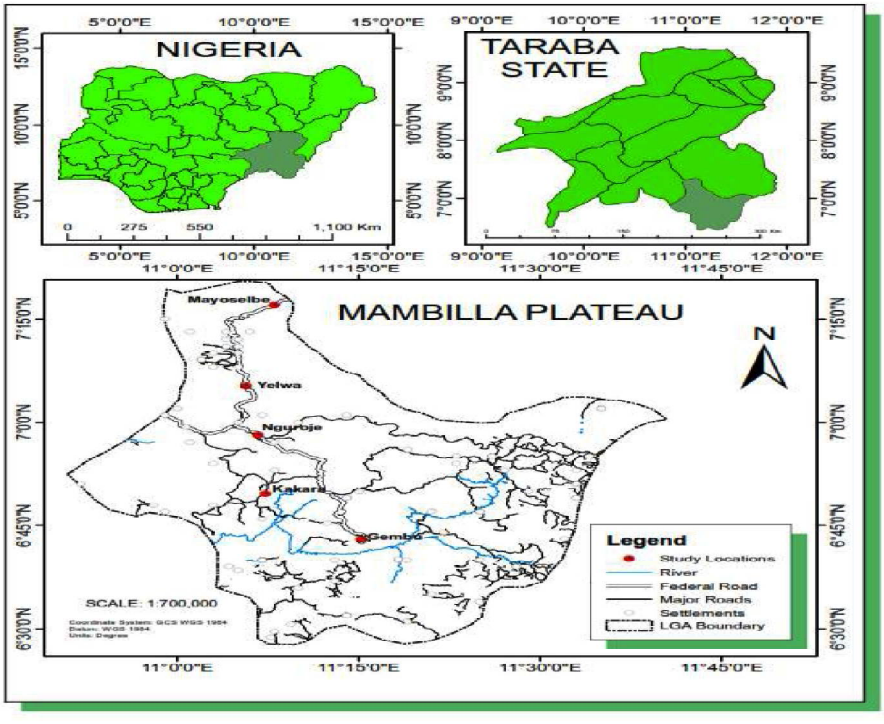

Figure 1: Map of Nigeria showing the study area

2.2 Study design

The study was carried out in five locations namely Nguroje, Yelwa, Gembu, Kakara and Mayo-selbe all located at different altitudes on the highlands of Mambilla Plateau Taraba State North East Nigeria.

2.3 Mosquito collections and preservation

2.3.1 Center for Disease Control (CDC) light trap collection

A traditionally defined cluster of 10 houses in each of the locations were chosen and three (3) houses equitably dispersed were randomly selected for inclusion in the sampling this gives a total of fifteen (15) houses in all. Mosquitoes were collected once every month. In every mosquito trapping night, light traps were positioned both inside and around the houses between 6pm in the evening and 6am in the morning. The traps were inspected every one hour. Members of *Anopheles* mosquitoes captured were aspirated into clearly labeled paper cups. Indoor traps were suspended from the ceiling at the foot end of the bed at approximately 1.5 m above the ground level in an occupied room (these were fixed stations for collection). The outdoor traps were hung on a post around the same houses.

2.3.2. Pyrethrum spray catches (PSCs)

Pyrethrum Spray Catches (PSC) were carried out from an average of eighteen (18) rooms where at least one inhabitant slept the previous night but in which indoor residual spraying was not done as this may interfere with the result of the pyrethrum spray catch. PSCs were carried out 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 min for mosquitoes to be knocked down. The knocked down mosquitoes were collected from the white sheets with forceps and put in clearly labeled petri dishes containing moist filter paper according to collection site, elevation and date of collection. The number of people sleeping in the room the night before collection period was also recorded. All the collected mosquitoes were taken to the laboratory for identification by microscopy. Female mosquitoes were classified according to their repletion status into unfed, fed, half-gravid and gravid specimens (WHO, 2009). Mosquito samples were preserved individually in an Eppendorf tube each on silica gel and taken to the laboratory for identification. They were later transferred to the Molecular Entomology and Parasitology Laboratory at the Nigerian Institute of Medical Research (NIMR), Lagos Nigeria for further analysis.

2.3.3. Larval collection and rearing

Larvae were collected using the standard dipping method (Service, 1993) from between 0.8 a.m. to 10.00 a.m. In all locations where larvae were sighted, larvae of all available instar were collected anopheline larvae were distinguished from culicines based on their resting habits in water and respiratory siphons. The edge of the dipper was submerged, dipped at about 45 degrees about an inch below the surface of the water quickly but gently, the dipper was moved along a straight line in the water. The stroke was ended just before the dipper was filled to avoid overflowing. The dipper was then gently raised out of the water without spilling the water and the larvae. Depending on the size of each larval habitat, 10-30 dips were taken at intervals along the edge for about 30 minutes at each larval habitat (Kenea *et al*., 2011). The mosquito larvae were then transferred into plastic containers along with the breeding sites water. The plastic containers were labeled according to type of habitat, location of collection and coordinates of habitat. Time and date of collection were also recorded. To rear the mosquitoes, larvae from the field were transferred into small white transparent plastic buckets. These were filled to two-thirds of their volume with the breeding site water. The mouths of the plastic buckets were covered with mosquito net. A small hole was made at the center of the net and plugged with cotton wool until adults emerged. Larvae were fed with baker's yeast (Service, 1993).

2.4. Morphological identification

All mosquitoes were identified to species level using the morphological keys of Gillies and De Meillon (1968) and Gillies and Coetzee (1987). The mosquitoes were stored dry on silica gel after morphological identification. Specimens that were positively identified as belonging to *An. gambiae s.l.* were further tested by Polymerase Chain Reaction (PCR).

2.5. PCR identification of members of Anopheles gambiae complex and the molecular forms of Anopheles gambiae s s

The wings or legs of all mosquitoes morphologically identified as *An. gambiae s.l.* were used for DNA extraction. The mosquitoes were differentiated to species level using PCR which was performed with universal and

species specific primers for the *An. gambiae s.l.* Molecular identification of *An. gambiae* species complex is based on the species specific nucleotide sequences in the ribosomal DNA (rDNA) intergenic spacers (IGS) following the procedure of Scott *et al*., (1993). Five sets of primers designed from the DNA sequences of the IGS region of *An. gambiae s.l.* rDNA were used in PCR for the member species identification. The sequence details of the primers are abbreviated, UN primer anneals to the same position on the rDNA sequences of all five species, GA anneals specifically to *An. gambiae sensu stricto* ME anneals to both *An. merus* and *melas*, AR to *An. arabiensis* and QD to *An. quadriannulatus*.

The PCR reaction mix of 12.5 μl contained 1X PCR buffer 1.25 μl of each of the four oligonucleotide triphosphates (dNTPs), 1.0 μl of each ligonucleotide primers and 0.5 μl of Taq. DNA polymerase enzyme and 1.0 μl of the genomic DNA was used as template for the amplification reaction. To improve specificity primers that were not found in the sampling location were excluded in the master mix and sterile double distilled water was used to make up the volume to 12.5 μl for a single sample using pre-mix and using individual constituents. Each of this constituent of the master mix was multiplied by the number of samples identified.

2.5.1 PCR protocol for An. gambiae complex

One leg or wing of each mosquito was placed in 1.0 μl centrifuge tubes and appropriately labeled. A mixture of the primers and 12.5 μl master mix was pipetted and added to the micro centrifuge tubes containing the one leg or wing from each of the mosquitoes. A positive control with PCR products of *An. gambiae* of the same primer set and a negative control without DNA template were included for each reaction mixture and this reaction was placed in the PCR machine and this was programmed for *An. gambiae s l*. The reaction mixture was centrifuge for 3 minutes at 14000 rpm in order to separate the template DNA from the tissues. The mixture was spun with the aid of a PTC 100 thermal cycler (MJ Research Inc., USA) according to the PCR conditions as follows; Initial Denaturation at 95 ^{o}C briefly for 2 minutes, 30 cycles each of denaturation at 95 ^{o}C for 30 seconds, annealing at 55 ^{o}C for 30 seconds, extension at 72 ^{o}C for 40 sec and final extension at 72 ^{o}C for 7 minutes. The amplified DNA was separated on a 1.0% agarose gel stained with ethidium bromide and viewed on a UV transilluminator.

2.5.2. PCR protocol for An. gambiae s.s.

For the identification of molecular forms of *An. gambiae s.s.* the constituents of the digest consist of 0.2 μl enzyme and 0.6 μl buffer, sterile double distilled water was used to make up the volume to1.8 μl for individual constituents. Protocol involved adding 1.8 μl of digest mix and 10 μl of PCR product to each tube and PCR was performed as in the case of *An. gambiae sl* and the product was run on gel as well.

2.6. Data analyses

Relative densities of species were calculated to compare the number of female anophelines captured in different altitude locations according to Bashar and Tuno (2014). Shannon wiener diversity index (H') Shannon (1948) was used to estimate species diversity of the mosquitoes caught at different study locations. Logistic correlation analysis was employed to study the relationship between molecular forms and altitude using SPSS® 16.0 (© SPSS Inc., Chicago, IL. 2007).

3. Results

3.1. Morphological identification of anophelines

A total of 878 anopheline mosquitoes comprising of five species were collected from across the five altitudinal locations. *An. gambiae s.l.,* was the most abundant of all the species collected that is; 757(86.22%), followed by *An. coustani* 73(8.31%), *An. funestus* 29(3.30%), 18(2.05%) and the least *An. rufipes* 1(0.11%). Mayo-selbe recorded the highest number of mosquitoes collected 572(65.15%), Gembu was next with 163(18.56%), Nguroje 69(7.86%), Yelwa 24(2.73%), Kakara had 50(5.69%) mosquitoes. Four out of the five anopheline mosquito species were caught in Yelwa, Gembu and Mayo-selbe while three species each were caught in Kakara and Nguroje each. The number and species composition of the *Anopheles* mosquitoes are represented in Table 1.

Adult *Anopheles* mosquitoes make up 79.61% of the total collection along the altitudinal locations. Table 2 shows the relative densities of the five anopheline species *An. gambiae* had the highest relative density of 93.42, *An. coustani* 5.01, *An. funestus* 0.86, *An. pharoensis* 0.57 while *An. rufipes* had the least 0.14. Table 3 reveal that in the dry season, December recorded 235 number of mosquitoes and this was the highest collection for the season. the species diversity was however low 0.28. *An. gambiae* had high dominance of 0.75 over other

Table 1: Number and species composition of *Anopheles* mosquitoes along altitudinal locations

		Species					
Locations	**Altitude (m)**	***An. gambiae***	***An. coustani***	***An. funestus***	***An. pharoensis***	***An. rufipes***	**Total**
Nguroje	1,885	66	2	0	0	1	**69**
Yelwa	1,674	17	4	2	1	-	**24**
Gembu	1,584	115	20	17	11	-	**163**
Kakara	1,496	34	12	4	0	-	**50**
Mayo-selbe	484	525	35	6	6	-	**572**
Total		**757**	**73**	**29**	**18**	**1**	**878**

Table 2: Relative densities of female anophelines along altitudinal locations

Species	**Locations**																	
	Nguroje			**Yelwa**			**Gembu**			**Kakara**			**Mayo-selbe**			**Grand Total**		
	Light Trap		PSC	Light Trap		PSC	Light Trap		PSC	Light Trap		PSC	Light Trap		PSC	N	RD	
	In	Out		In	Out		In	Out		In	Out		In	Out				
An. gambiae	3	3	3	7	2	8	11	4	48	1	0	12	68	11	472	653	93.42	
An. coustani	0	0	0	0	0	2	0	0	0	3	2	1	5	1	21	35	5.01	
An. funestus	0	0	0	0	0	0	0	0	0	0	0	0	2	1	3	6	0.86	
An. pharoensis	0	0	0	0	0	1	0	0	0	0	0	0	0	0	3	4	0.57	
An. rufipes	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.14	
Total	**3**	**4**	**3**	**7**	**2**	**11**	**11**	**4**	**48**	**4**	**2**	**13**	**75**	**13**	**499**	**699**	**100**	
Grand Total	**10**			**20**			**63**			**19**			**587**					

Table 3: Monthly species diversity of anopheline vectors of malaria

Months	**Species richness (S)**	**Species abundance (N)**	**Diversity index (H')**	**Maximum diversity (H_{max})**	**Evenness (J)**	**Dominance (D)**
November	4	95	0.59	1.39	0.42	0.58
December	3	235	0.28	1.10	0.25	0.75
January	2	58	0.10	0.69	0.14	0.86
February	2	55	0.17	0.69	0.25	0.75
March	1	6	0.00	0.00	0.00	1.00

Table 3 (Cont.)

Months	Species richness (S)	Species abundance (N)	Diversity index (H')	Maximum diversity (H_{max})	Evenness (J)	Dominance(D)
April	3	49	0.64	1.10	0.58	0.42
May	3	65	0.46	1.10	0.42	0.58
June	4	104	0.99	1.39	0.71	0.29
July	1	7	0.00	0.00	0.00	1.00
August	2	16	0.23	1.10	0.21	0.79
September	4	98	0.58	1.39	0.42	0.58
October	4	91	0.60	1.39	0.43	0.57

species. March recorded the least abundance with only six mosquitoes caught. The diversity index was 0 because only *An. gambiae* were caught in March therefore, the species had the highest dominance of 1.00 over other species. In the rainy season, 104 mosquitoes were collected in June there was also a high diversity index of 0.99 but *An. gambiae* had low dominance of 0.29 over other species.

For the locations, Table 4 shows that. Mayo-selbe had the highest species abundance of 572 but lowest species diversity index of 0.24 *An. gambiae* dominated over other anopheline species 0.76. Gembu recorded the next higher abundance with 163 mosquitoes it also recorded the highest diversity index of 0.99 as well as the

Table 4: Species abundance and diversity of anopheline vectors of malaria across the altitudinal locations

Location	Species richness (S)	Species abundance (N)	Diversity index (H')	Maximum diversity (H_{max})	Evenness (J)	Dominance (D)
Nguroje	3	69	0.61	1.10	0.55	0.45
Yelwa	4	24	0.81	1.39	0.58	0.41
Gembu	4	163	0.92	1.39	0.81	0.19
Kakara	3	50	0.81	1.10	0.74	0.26
Mayo-selbe	4	572	0.34	1.39	0.24	0.76

Table 5: Molecular forms of *An. gambiae s.s.* along altitudinal locations

Location	Number sampled	*Anopheles gambiae* Molecular Forms		Not identified	r^2
		M form	S form		
Nguroje	66	21	45	-	0.94204
Yelwa	17	5	12	-	
Gembu	115	15	83	17	
Kakara	34	11	23	-	
Mayo-selbe	525	140	357	28	
Total (%)	757	192(25.36)	520(68.69)	45(5.94)	

Note: r^2 = Correlation coefficient, a very strong positive correlation between the M and S forms. As the M forms increase along the locations so also the S forms.

lowest *An. gambiae* dominance of 0.14. Nguroje recorded 69 mosquitoes abundance and species diversity index of 0.61 *An. gambiae* dominance over other species was 0.45. Kakara had abundance of 50 and a high diversity index of 0.81 but *An. gambiae* recorded low dominance 0.26. Yelwa had the least abundance but very high diversity index of 0.81, *An. gambiae* dominance over other species was 0.41.

3.2. Molecular characterization (M and S) forms

Mosquitoes morphologically identified as *An. gambiae s.l.* species were further identified to species and *An. gambiae s.s.* molecular forms. Out of the 757 *An. gambiae s.l.* tested 712(94.06%) of these were identified as *An.*

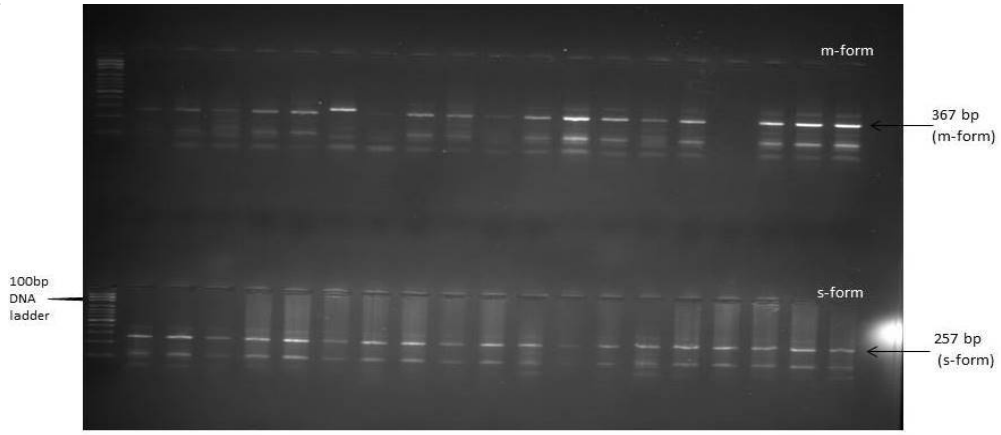

Figure 2: Amplified fragment using the species – specific assay for the identification of member of the *An. gambiae s.s.* Lane 1 lader (molecular marker), lane 2 negative control: lanes 3-20 test lanes of *An. gambiae s.s.* (m-form=367 bp and s-form=257 bp)

gambiae s.s. and they occurred in all the altitudinal locations and months of collection 45(5.94%) could not be identified this could have resulted from incorrect morphological identification, DNA degradation due to bad preservation or human error. Out of the 712 *An. gambiae s.s.,* 192(25.37%) were M form and 520(68.69%) were S form (Table 5).

4. Discussion

All the anopheline species reported in the current study have been reported in other parts of Nigeria and Africa (Okorie *et al*., 2011; Mala *et al*., 2011; Fornadel *et al*., 2011; Munhenga *et al*., 2014; and Dida *et al*., 2015). This confirms the wide range of geographic distribution of the anophelines. *An. gambiae s.l.* was the most abundant of all the anophelines species encountered it was recorded monthly in all the five altitude locations however, the populations were not abundant throughout the season. Several studies have reported similar high abundance of *An. gambiae s.l.* from different parts of Nigeria (Ayanda, 2009; Okorie, *et al*., 2011; Afolabi *et al*., 2013; and Aigbodion *et al*., 2013) it is among the most often reported malaria vector species in the country (Okorie *et al*., 2011). *An. gambiae* and *An. funestus* were two principal anopheline species identified in the current study this agrees with Ayanda, (2009) who recorded these two species out of the three *Anopheles* species recovered in Nassarawa state. *An. gambiae* and *An. funestus* complexes together comprised 14.4% of the total species recorded in Benin City Nigeria (Aigbodion *et al*., 2013), they form 65.2% and 17.3% respectively of most commonly reported malaria vectors in Nigeria (Okorie *et al*., 2011). Earlier study in other African highlands had reported these two species as the predominant anophelines (Bødker *et al*., 2003). This has serious health implications as the two species have been identified as important malaria and lymphatic filariasis vectors in Nigeria (Aigbodion *et al*., 2013) particularly so that Nigeria is one of the countries with high percentage of malaria prevalence in Sub-Saharan Africa (WHO, 2015 and 2017). *An. funestus* adapts well and has a wide geographic spread in Sub-Saharan Africa it has high affinity for human blood and feeds indoors. The species is suggested as more efficient in malaria transmission at some instances than *An. gambiae* (Sinka, 2013).

Several studies are suggestive of the fact that some *Anopheles* species which until now were not transmitting the disease are now assuming vector status (Mwangangi *et al*., 2013). *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 (Okorie *et al*., 2011), Zambia (Fornadel *et*

al., 2011), South Africa (Munhenga *et al*., 2014) Kenya and Tanzania (Mwangangi *et al*., 2013; and Dida *et al*., 2015) among others. In the current study, the species was not tested for *Plasmodium* sporozoites it was found negative for *Plasmodium* parasites in Southern Zambia (Fornadel *et al*., 2011) but contributed a substantially high Entomological Inoculation Rate (EIR) with 23.91 infectious bites per person per year to the overall EIR recorded in Kenya (Mwangangi *et al*., 2013) and was found to be playing a major role in outdoor transmission. The high demonstration of anthropophilic tendencies is suggestive of its potential as a secondary malaria vector in Africa (Fornadel *et al*., 2011) 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 country and may play a significant role in malaria transmission in the future.

An. rufipes and *An. pharoensis* were less abundant among all anopheline species encountered *An. rufipes* had the least abundance 0.11% and only in Nguroje this is in conformity with Munhenga *et al*. (2014) who recorded least abundance of *An. rufipes* 0.1% and confined to only one location of the five locations sampled. *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 (Norris and Norris, 2015).

In the dry season, December experienced more anophelines species abundance and least in March. December marked the beginning of the dry season when more larval breeding habitats were newly formed by the receding rains as well as high emergence of adult mosquitoes. March indicates the peak of the dry season and it was observed that most of the breeding sites were dried up thereby reducing adult abundance. Also most of the potential breeding sites were man-made and these dried up as the dry season intensified. This could explain the high anophelines species abundance in December and reduction in same during March. A similar trend was observed in the Usambara Mountains in Tanzania where highest anopheline mosquitoes were collected at the end of the long rains (Bødker *et al*., 2003). Shortly after rainfall, *Anopheles* mosquitoes tend to quickly recolonize habitats (Mala *et al*., 2011). In the rainy season, June and September recorded more anophelines abundances. June recorded the highest abundance and species diversity while least abundances were observed in July and August with July recording the least abundance and diversity of species. These differences could be as a result of the change in precipitation. In the study area, the rains were steady and moderate in June and September. Larval habitats were well-established and productive thereby resulting in more larval presence. The temperatures and relative humidity were also favorable to both the larvae and adult developments.

On the contrary, July and August indicated the peak of the rainy season and it was observed that most of the breeding sites were flooded and some overgrown with tall grasses. Larvae were washed away by the heavy rains thereby reducing the number that emerged to adults. This result agrees with earlier study in Bali Local Government Area of the same state in which *An. gambiae s. s.* were collected mostly in June and none during the peak rainy months of July and August (Lamidi *et al*., 2017). It is a general consensus that precipitation is associated with extended duration of water bodies for the female mosquitoes to successfully oviposit and larvae to develop to adults. It is also a known fact that larval habitats can become flooded with excessive precipitation resulting in reduced adult population density due to washing away of the larvae (Vajda *et al*., 2017).

The high species abundances recorded by Mayo-selbe and Gembu have serious epidemiological consequence. Mayo-selbe is at the foot of the mountain and has more favorable environmental conditions for both adult development and parasites development time within the vector. Mayo-selbe could serve as a reservoir of malaria transmission to the highlands. Gembu is strategic in the Mambilla plateau. It is the center of convergence of people from the lowlands and other smaller or more rural locations within the Mambilla Plateau. Highest species diversity index and lowest *An. gambiae* dominance were recorded in Gembu. Some of the mosquito species sampled from this location have been reported to play significant and some secondary roles in malaria transmission elsewhere (Mwangangi *et al*., 2013; and Sinka, 2013), these species could assume vector status in the future in the absence of *An. gambiae*.

In the current study, *An. gambiae* S form dominated over the M form a recent study in another part of the state however found the M form being dominant over the S form (Lamidi *et al*., 2017). This is to be expected as the S form is known to show preference to breed in temporary pools which are formed shortly after the rains while the M forms prefer habitats such as rice fields or flooded areas, which are more permanent in nature (Sogoba, *et al*., 2008; Sinka, 2013; and Coetzee *et al*., 2013). These two habitat types typify the nature of larval habitats found in the Mambilla Plateau and Bali respectively. Earlier study by Onyabe *et al*. (2003) found the two molecular forms to occur all over the country (Nigeria) irrespective of the ecological location. About 45(5.94%) could not be identified this could have resulted from incorrect morphological identification, DNA degradation due to bad preservation or human error.

Results of the current study revealed the composition of anopheline mosquito species in the study area. Population of *An. gambiae s.l.* was the most abundant of the five species of anopheline mosquito and *An. gambiae s.s.* was the main anopheline vector of malaria on the highlands of Mambilla Plateau Nigeria. This poses serious health implications as *An. gambiae* is the major vector of malaria in sub-Saharan Africa and Nigeria in particular where the burden of the disease is still high.

5. Conclusion

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 behavior which increases its vector-human contact 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 Nigeria and Africa as a mechanism to prevent future epidemics of malaria in the highland regions of the country, Mambilla Plateau in particular.

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