DISTRIBUTION AND ABUNDANCE OF *ANOPHELES* MOSQUITO SPECIES IN THREE SELECTED AREAS OF TARABA STATE, NORTH-EASTERN NIGERIA

[1]LAMIDI, Tajudeen Babatunde, [2]ALO, Emmanuel Babatope and [2]NAPHTALI, Rebecca

[1]Department of Science Laboratory Technology, Federal Polytechnic, PMB 05, Bali, Taraba State, Nigeria.

[2]Department of Zoology, Modibbo Adama University of Technology, PMB 2076, Yola, Adamawa State, Nigeria.

Corresponding Author: Lamidi, T. B. Department of Science Laboratory Technology, Federal Polytechnic, PMB 05, Bali, Taraba State, Nigeria. **Email:** tajudeenlamidi@hotmail.com **Phone:** +234 8063837972

ABSTRACT

Most mosquitoes are of public health problem because the females consume blood and transmit diseases. The study was carried out between September 2015 and August 2016 to determine the diversity of Anopheles species in Taraba State, Nigeria. Indoor mosquitoes in Ardo Kola, Bali and Donga were collected using pyrethrum-spray catch. 1073 Anopheles mosquitoes were identified to 20 species level. The identified Anopheles gambiae species complex further analyzed to determine sibling and molecular species using PCR-Restriction Fragment Length Polymorphism assay contained An. coluzzii (former An. gambiae ss M form) 725(95.6 %) An. gambiae (former An. gambiae ss S form) 24(3.2 %), An. coluzzii/An. gambiae hybrid 9(1.2 %) and An. arabiensis 1(0.19 %). Among the Anopheles species, 640(59.6 %) which composed of 14 species were from Ardo Kola, 305(28.4 %) composed of 15 species were from Bali and 128(12.0 %) composed of 7 species were from Donga, all dominated by An. coluzzii which was 66.4, 21.2 and 12.4 % respectively of all An. coluzzii, **followed by** ***An. constani*** **in Ardo Kola and** ***An. rivulorum*** **in Bali and Donga*****. An. gambiae, An. gambiae M/S hybrid and An. arabiensis were only found in Bali 24(100.0 %), 9(100.0 %) and 1(100 %) respectively. An. pariensis was only found in Donga 1(100.0 %). The distribution of Anopheles in months did not appear to follow a particular pattern, although observed to be generally low in dry months. Analysis of variance showed significant difference in abundance of Anopheles species in study areas (p<0.05). This means that the inhabitants of the study areas are, all year round at risk of mosquito borne-diseases.***

Keywords: Abundance, *Anopheles* species, Sympatric speciation, Vector ecology, Guinea-savanna, Nigeria

INTRODUCTION

Mosquitoes of the family Culicidae are considered a nuisance and a major public health problem, because their females feeds on human blood and thus transmit extremely harmful diseases, such as malaria, yellow fever and filariasis (Wikipedia, 2014). They are estimated to transmit diseases to more than 700 million people annually and responsible for the death of about 1 in 17 people (WECM and WHO, 2000). Effective transmission of mosquito-borne disease requires successful contact between female mosquitoes and their hosts (Xu *et al*., 2014). Among Anophelinae, the members of the genus *Anopheles* are best known for their role in transmitting malaria and filariasis worldwide (Service, 2008; WHO, 2013). Of these diseases, malaria caused by *Plasmodium* parasite is one of the greatest killer diseases in the world (WHO, 2013). WHO (2013) reported an estimated 207 million cases of malaria in 2012 out of which

200 million cases (80.0 %) were in Africa continent. The distribution pattern, transmission and intensity of the disease are dependent on the degree of urbanization and the distance from vector breeding sites (CDC, 2015). The endemicity of malaria in any region is determined by indigenous *Anopheles* mosquitoes, abundance, feeding, resting behavior and their *Plasmodium* infectivity, among other factors (Molta, 2000; WHO, 2003).

Federal Ministry of Health, Abuja reported that at least 50.0 % of Nigerians suffered from one form of malaria or the other making it the most significant health problem in Nigeria (Chukwuocha, 2012). The high transmission rate and prevalence of malaria is a result of the diverse mosquitoes breeding sites, which include practically receptacle that holds water, such as tins, cans, old tyres, tree holes, cisterns, open pools, drainage, stream and pond (Ingstad *et al*., 2012). Part of the efforts being made is the official commemoration of April 25 every year, starting from 2008 as World Malaria Day (CDC, 2017)

People living in poor rural areas are confronted with a multitude of barriers when assessing malaria prevention especially on the knowledge of the biology and ecology of the vectors, among others (Ingstad *et al*., 2012. The mapping of malaria vectors is important in the control of malaria. This is because the species composition and distribution and other biological parameters of the mosquitoes are poorly known in different ecological zones of Nigeria and in most of the malaria endemic areas due to the difficulties in the morphological identification of certain complex species, the knowledge of which is required in the design of vector control programmes and in tackling the prevalence of the disease in endemic areas (Awolola *et al*., 2002). Taraba State of Nigeria is endemic to malaria with reported prevalence of 80.0 % in some areas of the state (TSMH, 2010).

According to the World Health Organization, malaria is still the major cause of death in children in sub-Saharan Africa. The disease in this region takes the life of a child every 2 minutes (WHO, 2015).

Therefore, the aim of this study was to investigate the distribution and relative abundance of *Anopheles* mosquitoes in major riverine communities of selected Local government areas of Taraba State, Nigeria covering the seasons of the year.

MATERIALS AND METHODS

Study Area: Taraba State is located between longitude 8.5° – 11.6°E and latitude 6.5° – 9.5°N (8° 00'N and 10° 3O'E coordinates) in the north-eastern geopolitical zone of Nigeria with a size of 54,473 square kilometers representing 5.89 % of the country landmass (Wikipedia, 2015). It has an estimated population of 2,688,944 based on 2006 census, giving a population density of 27 people per km^2, representing 1.90% of the total population of Nigerians (Wikipedia, 2015).

The study was carried out in three major riverine communities: Mayorenewa, Bali and Gyatan-aure located in three Local Government Areas of Taraba State, Nigeria. These are Ardo Kola (northern zone, 8° 40' – 9° 12' N; 10° 58' – 11° 33'E), Bali (central zone, 7° 22'- 8° 48'N; 10° 17' – 11° 49'E) and Donga (southern zone, 7° 15' – 7° 56'N; 9° 47'- 10° 42'E) respectively (Figure 1).

The study communities were selected based on dense population, house types, presence of water bodies, both permanent slow running ones and stagnant prevailing pools of water that serve as breeding places for mosquitoes.

Taraba State is in guinea savanna (semi-arid) zone of the country. Rainy season is between May and early October and dry season between November and April. Daily temperature varies from 37° to 40°C during the hottest months of March/April. It also varies from 32° to 37°C during the coldest months of December/January. The relative humidity is about 23.00 % during the hot dry weather and can reach 80.00 % during the peak of wet season in July/August (Dammo *et al*., 2015).

Mosquito Sampling: Indoor mosquito collections were carried out every month of the study period, September 2015 to August 2016 in each of the areas.

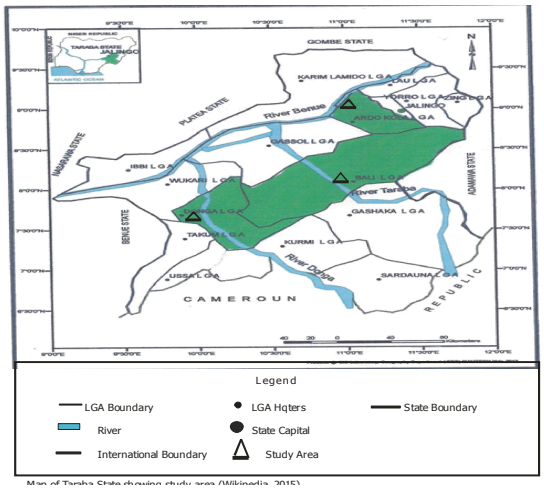

Figure 1: Map of Taraba State, Nigeria showing the study area (TSMH, 2010)

Ten bedrooms were selected randomly from each station with at most three bedrooms from the same house. 360(12 months x 10 bedrooms x 3 stations) rooms were sprayed during the entire period of study.

Collection of mosquitoes by use of non-residual insecticide-pyrethrum (Spread Sheet Collection) was employed using World Health Organization (WHO) standard procedure (WHO, 1995; WHO, 2003) and complemented with electronic mosquito bait. All knocked down mosquitoes were collected into properly labeled Eppendorf tubes and preserved over silica gel.

Laboratory Examination of Mosquitoes: Collected and preserved mosquitoes were brought to the laboratory to be sorted, morphologically identified to species level (Gillett and Smith, 1972; Gillies and Coetzee, 1987) before further processing.

Morphological Identification and Sorting out of Mosquitoes: Anopheline were separated from Culicine mosquitoes according to the morphological characteristics of their maxillary palps and identified mosquito genera were sexed based on the presence or absence of plumose (feathery) antennae. The morphological identification of different species of female *Anopheles* was done by studying the scales and colour of the palps at the head region, the patterns of spots on the wings, thorax, terminal abdominal segments, scales of the legs using dissecting microscope following the taxonomic keys of Gillett and Smith (1972) and Gillies and Coetzee (1987). The wings and legs of the *Anopheles* gambiae were separately preserved in PCR tube for molecular identification of the sibling species.

Molecular Identification of Species in the *Anopheles gambiae* Complex: The multiplex PCR technique was used for molecular characterization of members of the *An. gambiae* complex as described by Okwa *et al*. (2007), Brogdon and Chan (2010) and Kabbale *et al*. (2016). Direct method was used where legs and (or) wings of each morphologically identified member of *An. gambiae* complex were placed in PCR tube/sample templates covered with reaction mixture. A 12.5 ul reaction mixture was prepared. This contained 4 ul of master mix (DNA polymerase, reaction buffer, 7.5 ml $MgCl_2$ and 1 ml of dNTPs), gently vortexed for homogenization and briefly centrifuged after thawing, 6.15 ul of deionized distilled water and 0.5 ul of multiple sets of species-specific primers (*An. melas, An. gambiae ss, An. arabiensis*) and 0.25ul of *An. quadriannulatus* primers and universal primer for the amplification of several targets in a single PCR experiment of each *Anopheles* complex (Kabbale *et al*., 2016). Thereafter, 12.5 ul of reaction mixture was added to each of the mosquito samples in the PCR tubes. Each of the tubes was loaded in the PCR machine and programmed. Samples were run on a rotor-gene 6000 using the temperature cycling conditions as follows: Heated lid at 110^{O}C and initial denaturation at 95^{O}C for 3 minutes, followed by 30 cycles of denaturation at 95^{O}C for 30 seconds, annealing at 55^{O}C for 30 seconds and extension at 72^{o}C for 40 seconds, then followed by final extension at 72^{O}C for 7 minutes and final hold at 8^{o}C.

Preparation of 1.5 % Agarose Gel and Loading for Electrophoresis: 3 g of agarose gel was added to 200 ul of TBE (Tris HCl salt, Buric acid, EDTA). It was heated till the gel dissolved and allowed to cool down at room temperature. 10 ul of ethidium bromide was added to the gel. Thereafter the gel was cast into the trough, where it was allowed to solidify.

The trough was moved into a tank filled with TBE buffer. After harvesting samples from thermocycler, 10 ul of each PCR products was loaded directly into each well on the casted gel. Laboratory strains (standard specimens) of the *Anopheles* complexes were used as positive control in different lanes/control template. 1kb DNA marker (ladder) was used as standard bands in first and second to the last lanes. Negative control template contained neither the target sample nor the standards at last lanes. The amplified DNA was separated on a 1.5 % agarose gel electrophoresis stained with ethidium bromide at 80 volts for 50 minutes. The amplified fragment was visualized on an ultraviolent (UV) transilluminator (gel documentation system). The size of the bands of the separated DNA was matched with the standard specimen (positive controls) from where the sibling species of the target samples were identified. The PCR consumables/reagents, primers, markers and standard specimens for positive control were acquired with the assistance of Nigeria Institute of Medical Research, Yaba Lagos State.

Molecular Genotyping of Identified *Anopheles gambiae sensu stricto:* PCR-restriction fragment length polymorphism (RFLP)-based analysis is a popular technique for genotyping or determining genetic variants of a species, in this case identified *An. gambiae ss*. It exploits variations in homologous DNA sequences. 1.8 ul of digest mix (1 ul distilled water, 0.6 ul buffer, 0.2 ul restriction enzyme) was added to each tube and 10 ul of PCR product identified as *An. gambiae ss* was added to each tube. The samples were subjected to thermocycling using PCR for 1 hour 20 minutes. After harvest, 10 ul of PCR product was loaded into each well of the prepared gel with standard marker, positive and negative control. The PCR product was ran for 1 hour at 80 volts and gel documentation photograph was taken from Gel documentation system using UV light (Rasmussen, 2012; Kabbale *et al*., 2016).

Data Analysis: The data obtained were subjected to descriptive statistics; percentages were calculated and presented in tables. Analysis of variance (ANOVA) was used to determine the significant difference ($p<0.05$) between *Anopheles* mosquito abundance in relation to study areas using SPSS.

RESULTS

Distribution and Relative Abundance of Mosquito Genera in Study Areas: A total of 3,369 indoor resting mosquitoes belonging to six genera: *Anopheles* 1686(50.0 %), *Culex* 1587(47.1 %), *Aedes* 5(0.2 %), *Mansonia* 67(2.0 %), *Sabethes* 18(0.5 %) and *Toxorhynchites* 6(0.2 %) were collected. Mosquito abundance in relation to study areas showed that Ardo Kola had the highest 1180(35.0 %), followed by Bali 1107(32.9 %) and Donga 1082(32.1 %). Analysis of variance showed significant difference in mosquito genera in relation to study areas ($p<0.05$) (Table 1).

Distribution and Relative Abundance of *Anopheles* species in Study Areas: The distribution of identified members of the *Anopheles* species by studied sites indicated that the 1073 *Anopheles* mosquitoes identified to species level contained 20 species thus: *Anopheles coluzzii* (former *An. gambiae* M) 724(67.5 %), followed by *An. rivulorum* 124(11.5 %), *An. constani* 86(8.0 %), *An. salbaii* 37(3.4 %), *An. gambiae* (former *An. gambiae* S) 24(2.2 %), *An. rufipes* 21(1.9 %), *An. garhami* 10(0.9 %), *An. gambiae* M and S hybrid 9(0.8 %), *An. aruni* 7(0.6 %), *An. maculipalpis* 6(0.5 %), *An. pharoensis*, *An. rhodensiensis* and *An. funestus* 4(0.4 %), *An. tenebrosus* 3(0.3 %), *An. caliginosus*, *An. concolor* and *An. nili* 2(0.1 %), *An. hancoki*, *An. arabiensis* and *An. pariensis* 1(0.1 %) (Table 2). Among the 1073 identified *Anopheles* species, 640(59.6 %) which composed of 14 species were from Ardo Kola and dominated by *An. coluzzii* which was 481(66.4 %) of all *An. coluzzii* identified from all the study areas. *An. rufipes* was only present in Ardo Kola 21(100.0 %). Among the 1073 identified *Anopheles* species, 305(28.4 %) composed of 15 species were from Bali and dominated by *An. coluzzii* which were 154(21.2 %) of all *An. coluzzii* identified.

Table 1: Distribution and abundance of morphologically identified mosquito genera in relation to study areas in Taraba State, Sept 2015 to August 2016

Mosquito genera	Study Area			Total
	Ardo Kola	Bali	Donga	
Anopheles	1017(60.3)[a]	409(24.3)[b]	260(15.4)[c]	1686(50.0)
Culex	130 (8.2)[a]	688(43.4)[b]	769 (48.5)[c]	1587(47.1)
Aedes	0(0.0)[a]	2(40.0)[b]	3(60.0)[c]	5(0.2)
Mansonia	11(16.4)[a]	8 (11.9)[b]	48(71.6)[c]	67(2.0)
Sabethes	18(100)[a]	0(0.0)[b]	0(0.0)[b]	18(0.5)
Toxorhynchites	4(66.7)[a]	0 (0.0)[b]	2(33.3)[c]	6(0.2)
Total	1180(35.0)	1107(32.9)	1082(32.1)	3369

Figure in parenthesis =%, Number and percentages on the same row with different alphabet superscript are significantly different (p<0.05)

Table 2: Distribution of identified female *Anopheles* species in relation to study area

Mosquito species	Study Areas			Total
	Ardo Kola	Bali	Donga	
Anopheles coluzzii	481(66.4)[a]	154 (21.2)[b]	90(12.4)[c]	725(67.5)
An. gambiae	0(0.0)[a]	24 (100)[b]	0 (0.0)[a]	24 (2.2)
An. gambiae M/S	0(0.0)[a]	9 (100)[b]	0 (0.0)[a]	9 (0.8)
An. pharoensis	3 (75.0)[a]	1(25.0)[b]	0 (0.0)[c]	4 (0.4)
An. garrhami	7(70.0)[a]	3 (30.0)[b]	0 (0.0)[c]	10 (0.9)
An. rufipes	21(100.0)[a]	0 (0.0)[b]	0 (0.0)[b]	21(1.9)
An. salbaii	33(89.2)[a]	2 (5.4)[b]	2 (5.4)[b]	37 (3.4)
An. caliginosus	0(0.0)[a]	1(50.0)[b]	1 (50.0)[b]	2 (0.2)
An. concolor	1(50.0)[a]	1(50.0)[a]	0 (0.0)[b]	2 (0.2)
An. constani	50(58.2)[a]	34(39.5)[b]	2 (2.3)[c]	86 (8.0)
An. rhodensiensis	4(100.0)[a]	0(0.0)[b]	0 (0.0)[b]	4 (0.4)
An. maculipalpis	3(50.0)[a]	3 (50.0)[a]	0 (0.0)[b]	6 (0.5)
An. rivulorum	29(23.4)[a]	67 (54.0)[b]	28 (22.6)[c]	124 (11.5)
An. aruni	3(42.9)[a]	0 (0.0)[b]	4 (57.1)[c]	7 (0.6)
An. funestus	3(75.0)[a]	1(25.0)[b]	0 (0.0)[c]	4 (0.4)
An. tenebrosus	0 (0.0)[a]	3 (100)[b]	0 (0.0)[a]	3 (0.3)
An. hancoki	1(100.0)[a]	0 (0.0)[b]	0 (0.0)[b]	1 (0.1)
An. nili	1(50.0)[a]	1(50.0)[a]	0 (0.0)[b]	2 (0.2)
An. arabiensis	0(0.0)[a]	1 (100)[b]	0 (0.0)[a]	1(0.1)
An. pariensis	0(0.0)[a]	0 (0.0)[a]	1 (100)[b]	1(0.1)
Total	640 (59.6)	305 (28.4)	128 (12.0)	1073

Figure in parenthesis =%, Number and percentages on the same row with different alphabet superscript are significantly different (p<0.05)

Table 3: Monthly distribution of identified *Anopheles* species in Ardo kola

Species	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Total
An. coluzzii	22	47	14	29	30	75	38	58	61	45	25	37	481(75.1)
An. pharoensis	2	0	0	0	0	0	0	0	0	1	0	0	3(0.5)
An. garrhami	2	0	0	0	1	0	2	0	1	0	1	0	7(1.1)
An. rufipes	1	0	0	2	0	0	1	5	11	1	0	0	21(3.3)
An. salbaii	3	1	1	3	0	3	11	0	10	1	0	0	33(5.1)
An. concolor	1	0	0	0	0	0	0	0	0	0	0	0	1(1.0)
An. constani	0	4	4	5	0	7	1	11	11	2	0	5	50(7.8)
An. rhodensiensis	0	2	0	0	2	0	0	0	0	0	0	0	4(0.6)
An. maculipalpis	0	0	0	0	0	0	0	0	0	3	0	0	3(0.5)
An. rivulorum	0	0	0	0	0	0	0	0	0	8	14	7	29(4.5)
An. aruni	0	0	0	0	0	0	0	0	0	3	0	0	3(0.5)
An. funestus	0	0	2	0	0	0	0	0	0	1	0	0	3(0.5)
An. hancoki	0	0	0	0	0	0	0	0	1	0	0	0	1(0.2)
An. nili	0	0	0	0	0	0	0	0	0	0	0	1	1(0.2)
Total	31 (4.8)	54 (8.4)	19 (3.0)	41 (6.4)	33 (5.1	85 (13.2	53 (8.3)	74 (11.5	95 (14.8	65 (10.1	40 (6.2)	50 (7.8	640

Figure in parenthesis =%

An. gambiae (molecular S form) and *An. gambiae* hybrid (molecular M+S forms) were only found in Bali 24(100.0 %) and 9(100.0 %) respectively. Among the 1073 identified *Anopheles* species, 128(12.0 %) composed of 7 species were from Donga and was also dominated by *An. coluzzii* which were 90(12.4 %) of all *An. coluzzii* identified. *Anopheles pariensis* was only found in Donga 1(100.0 %).

Monthly Distribution and Relative Abundance of *Anopheles*: Monthly distribution of *Anopheles* species in Ardo Kola showed that *Anopheles* mosquitoes were most abundant in the months of May 95(14.8 %), February 85(13.2 %) and April 74(11.5 %). Indoor resting *Anopheles* were least recorded in the months of November 19(3.0 %), September 31(4.8 %) and July 40(6.2 %). The predominant *Anopheles* species throughout the year were *An. coluzzii* 481(75.1 %) followed by *An. constani* 50(7.8 %), *An. salbaii* 33(5.1 %) and *An. rivulorum* 29(4.5 %), while the least observed were *An. hancoki* 1(0.2 %), *An. nili* 1(0.2 %), *An. maculipalpis* 3(0.5 %), *An. aruni* 3(0.5 %), *An. funestus* 3(0.5 %), *An. pharoensis* 3(0.5 %), *An. rhodensiensis* 4(0.6 %), *An. garhami* 7(1.1 %) and *An. rufipes* 21(3.3 %) (Table 3). Monthly distribution of *Anopheles* species in Bali showed that *Anopheles* mosquitoes were most abundant in the months of July 72(23.6 %), June 69(22.6 %) and August 36(11.8 %). The predominant *Anopheles* species throughout the year were *An. coluzzii* 154(50.5 %) followed by *An. rivulorum* 67(22.0 %), *An. constani* 34(11.4 %) and *An. gambiae* 24(7.9 %), while the least observed were *An. nili* 1(0.3 %), *An. arabiensis* 1(0.3 %), *An. pharoensis* 1(0.3 %), *An. caliginosus* 1(0.3 %), *An. concolor* 1(0.3 %), *An. funestus* 1(0.3 %), *An. funestus* 1(0.3 %), *An. salbaii* 2(0.6 %), *An. funestus* 1(0.3 %), *An. maculipalpis* 3(1.0 %) and *An. tenebrosus* 3(1.0 %) (Table 4).

Monthly distribution of *Anopheles* species in Donga study area showed that *Anopheles* mosquitoes were most abundant in the months of February 27(21.1 %), March 23(18.0 %) and June 21(16.4 %). The predominant *Anopheles* species throughout the year were *An. coluzzii* 90(70.3 %) followed by *An. rivulorum* 28(21.9 %) and *An. aruni* 4(3.1 %), while the least observed were *An. caliginosus* 1(0.8 %), *An. pariensis* 1(0.8 %), *An. constani* 2(1.6 %) and *An. salbaii* 2(1.6 %) (Table 5).

Molecular Forms of *Anopheles gambiae* Complex: Out of a total of 759 *Anopheles gambiae s.l.* samples 99.9 % (n =758) were all identified as *Anopheles gambiae sensu stricto* and spread across the study areas. *Anopheles arabiensis* 1(0.1 %) was found in Bali. .Out of 758 *An. gambiae s.s.* samples analyzed 758 (95.5 %) were identified as *Anopheles coluzzii* (formerly Mopti (M) form), 24 (3.2 %) were identified as *Anopheles gambiae s.s.*Giles or *Anopheles gambiae* (formerly Savannah (S) form) and 9 (1.2 %) were identified as *Anopheles coluzzii/Anopheles gambiae* hybrid (M/S form). *Anopheles gambiae* (Molecular S form) and *Anopheles gambiae* hybrid (Molecular M/S forms) were only found in Bali (Table 6).

DISCUSSION

The abundance of *Anopheles* species in Ardo Kola area could be attributed to a number of factors, one of which is that some communities in Ardo Kola are located along the bank of River Benue and experiences seasonal flooding which usually provides favourable temporary and permanent breeding sites for *Anopheles* and the area being also located north of Taraba State with ambient temperature that favours larval development (Service, 2004).

The predominance of *An. coluzzii* is in line with the findings of Aju-Ameh *et al*. (2016) in Benue state and that of Oduola *et al*. (2016) in Kwara State and the occurrence of *An. constani* as a major *Anopheles* in this study is similar to findings of Anosike *et al*. (2007) in Imo state. The abundance of these species of mosquito is attributed to the fact that these mosquito species are associated with human dwellings with indoor resting habits and the species are mainly anthropophilic (Oyewole *et al*., 2005). *Anopheles nili* was the least abundant; perhaps it is more common in humid/ neighboring montane zone of the study area (Wanji *et al*., 2003).

Table 4: Monthly distribution of identified *Anopheles* species in Bali

Species	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Total
An. coluzzi	8	6	8	0	9	3	11	12	10	38	31	18	158(50.5)
An. gambiae S	3	2	2	1	1	1	1	2	0	11	0	0	24()7.9
An. gambiae M/S	0	0	1	0	1	1	1	1	0	4	0	0	9(2.9)
An. pharoensis	1	0	0	0	0	0	0	0	0	0	0	0	1(0.3)
An. garrhami	0	0	0	0	0	0	1	1	1	0	0	0	3(1.0)
An. salbaii	2	0	0	0	0	0	0	0	0	0	0	0	2(0.6)
An. caliginosus	1	0	0	0	0	0	0	0	0	0	0	0	1(0.3)
An. concolor	0	0	0	0	0	0	0	0	1	0	0	0	1(0.3)
An. Constani	0	0	0	0	0	0	0	2	4	4	20	4	34(11.4)
An. maculipalpis	0	0	0	0	0	0	0	0	0	3	0	0	3(1.0)
An. rivulorum	0	0	0	0	0	0	7	11	6	9	21	13	67(22.0)
An. funestus	0	0	0	0	0	0	1	0	0	0	0	0	1(0.3)
An. tenebrosus	1	0	0	0	0	0	0	1	0	0	0	1	3(1.0)
An. nili	1	0	0	0	0	0	0	0	0	0	0	0	1(0.3)
An. arabiensis	1	0	0	0	0	0	0	0	0	0	0	0	1(0.3)
Total	18 (5.9)	8 (2.6)	11 (3.6)	1 (0.3)	1 1(3.6)	5 (1.6)	22 (7.2)	30 (9.8)	22 (7.2)	69 (22.6)	72 (23.6)	36 (11.8)	305

Figure in parenthesis =%

Table 5: Monthly distribution of identified *Anopheles* species in Donga

Species	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Total
An. coluzzii	2	1	5	8	7	10	23	0	3	12	11	8	90(70.3)
An. salbaii	0	0	0	0	0	2	0	0	0	0	0	0	2(1.6)
An. caliginosus	0	0	0	0	0	1	0	0	0	0	0	0	1(0.8)
An. constani	0	0	0	0	0	1	0	0	0	0	1	0	2(1.6)
An. rivulorum	0	0	0	0	0	11	0	0	0	6	7	4	28(21.9)
An. rruni	0	0	0	0	0	1	0	0	0	3	0	0	4(3.1)
An. pariensis	0	0	0	0	0	1	0	0	0	0	0	0	1(0.8)
Total	2 (1.6)	1 (0.8)	5 (3.9)	8 (6.2)	7 (5.5)	27 (21.1)	23 (18.0)	0 (0.0)	3 (2.3)	21 (16.4)	19 (14.8)	12 (9.4)	128

Figure in parenthesis =%

Table 6: **Distribution of molecular species of *An. gambiae sensu lato* in study area**

Anopheles gambiae **s.l. molecular forms**	**Study Area**			**Total**
	Ardo Kola	**Bali**	**Donga**	
Anopheles coluzzii	481(66.4)[a]	154(21.2)[b]	90(12.4)[c]	725(95.5)
An. gambiae	0 (0.0)[a]	24(100)[b]	0 (0.0)[a]	24(3.2)
An. gambiae M/S	0(0.0)[a]	9(100)[b]	0(0.0)[a]	9(1.2)
An. arabiensis	0(0.0)[a]	1 (100)[b]	0(0.0)[a]	1(0.1)
Total	481(63.4)	188(24.8)	90(11.8)	759

Figure in parenthesis =%, Number and percentages on the same row with different alphabet superscript are significantly different ($p<0.05$)

The distribution of mosquito in relation to the months/seasons of the year showed variations. Although it did not appear to follow a particular pattern, it was observed to be generally low in dry season (December – February) in all the study areas, when most of the temporary habitats are dried which is in line with findings of Bagoro *et al*. (2014).

In Ardo Kola the *Anopheles* species peaked at the end of dry season and at the onset of wet season and the least was at the end of wet season and in dry months. In Bali, the *Anopheles* species population was highest in the middle of wet season (June, July and August) but lowest in the end of wet season and dry months (December, October and February). The highest peaks of the female anopheline species in February and the smallest peaks during September, as observed in Ardo Kola study area was in line with findings of Bagoro *et al*. (2014). Combination of trends of *Anopheles* abundance in both Ardo Kola and Bali was observed in Donga in which *Anopheles* species, had the highest population observed in some dry months (February, March), in the middle of wet month (June) and the lowest at

the end of wet months (September and October).

The fact that the mosquito abundance coincides to a great extent with period of rainfall is an indication that rainfall plays significant role in mosquito population dynamics. This implies that in all the study areas, abundant rainfall and duration, temperature and relative humidity are factors that determine abundance of mosquito due to availability of breeding sites (permanent or temporary or both) throughout the year. Increase in human development, construction and agriculture activities generate diverse larval habitats which account for mosquito abundance in the study areas. This is similar to findings by Lamidi (2009) in Sahel region of Nigeria. Ototo *et al.* (2015) had earlier reported that human activities and behaviours of mosquito of different species affected the population of indoor-resting mosquitoes, among which are exposures of mosquitoes to insecticides, use of insecticide treated bed net and exophilic/endophilic behaviours of mosquitoes. However in some cases it was observed generally that the abundance of mosquito was not strongly dependent on rainfall as in Kwara State (Oduola *et al.,* 2016) where the mosquito peaked in April and as generally observed in this study.

Sibling species composition of *An. gambiae* complex in the study areas showed fresh water species of *An. gambiae sensu stricto* and *An. arabiensis* occurring in sympatry. *An. gambiae* ss were found predominantly in all areas and all seasons. The peak values were in February and March in Donga, April and May in Ardo Kola and June, July in Bali. In the findings of Ototo *et al.* (2015), *An. gambiae* peaked in April/May similar to that in Ardo Kola. These peaks were reached generally one month after the onset of the wet seasons which was reportedly the period of highest biting rate and possibly, among other factors, highest malaria transmission season (WHO, 2003; Ototo *et al*., 2015).

Relative Abundance of *An. gambiae* ss over *An. arabiensis* and their sympatric occurrence in this study is in line with findings of Awolola *et al*. (2002), Oyewole *et al*. (2005), Ebenezer *et al.* (2014) and Oduola *et al*. (2016).

The molecular species or genotypes of *An. gambiae ss* found in the study area were of Mopti (M), Savanna rDNA (S) types/strains and M and S hybrid strain.

An. gambiae ss (molecular M) now called *An. coluzzii* were predominant in all *Anopheles* species and genetic variants of *An. gambiae sensu stricto* and in all study areas. *Anopheles coluzzii*, among the genetic variants of *An. gambiae ss*., was followed by *An. gambiae ss* (S forms), now called *An. gambiae* and *An. gambiae* M+S hybrid, all of which occurred in sympatry. Sympatric occurrence of molecular species of *An. gambiae ss* had been reported in Kwara and Benue States of Nigeria by Oduola *et al.* (2016) and Aju-Ameh *et al.* (2016) respectively. However, none had reported occurrence of *An. gambiae* M+S hybrids in Northern Nigeria The only report of the occurrence of 1(0.5 %) of *An. coluzzii/An. gambiae* hybrid was in forest zone of Ibadan, Western Nigeria by Okorie *et al*. (2015). However evidence of hybridization resulting from laboratory crossing of *An. coluzzii* and *An. gambiae* had been reported by Ranson *et al*. (2000).

This finding is a foremost showing species composition and abundance of *An. gambiae sensu lato* and genetic variants of the *An. gambiae ss* based on seasons and locations not only in Taraba state and north-eastern Nigeria, but generally in Nigeria. In the three study areas, only *Anopheles coluzzii* were common to all. *An. gambiae* and *Anopheles* M+S hybrids were only found in Bali, Central zone of the state.

The occurrence of only 9(1.2%) of *An. coluzzii/An. gambiae* hybrid of all molecular species of *An. gambiae ss* in the study areas suggests restricted gene flow and therefore very high degree of reproductive isolation between the two species as also found in Ibadan by Okorie *et al.* (2015) with 0.5% occurrence among other molecular siblings.

Conclusion: The diversity of *Anopheles* mosquito species found indoor in the study areas and the hybridization of *An. gambiae* and *An. coluzzii* in one of the area as an adaptation to the environment are of public health concern.

This calls for further investigation and review of policies and management strategies based on emerging knowledge in these localities

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