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Studies on the composition and distribution of the different sibling species of *Anopheles gambiae* complex within Katagum area in Bauchi state, Nigeria

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Abstract
A study of the adult indoor resting mosquitoes was conducted to identify the different sibling species of *Anopheles gambiae* s.l., their abundance and distribution in Katagum area of Bauchi State, Nigeria. A total of two hundred (200) females mosquitoes were collected from June to December, 2016 in the study area. Out of these, 40 (20%) were Anophelines while 160 (80%) were Culicines. The month of July recorded the highest number of mosquitoes with peak values of 17 and 62 for the *An. gambiae* s.l. and the culicine species respectively. Then there after the number of both species of mosquitoes started to decline. However, in December no mosquito species was recorded at all from the sampling sites. Results from the molecular identification (PCR) of the different sibling species of the *An. gambiae* s.l. collected from the study areas, revealed that *An. gambiae* s.s. and *An. arabiensis* were the only two sibling species of the *An. gambiae* complex were recorded during this period of study. These two sibling species were found to occur together in all the sampling sites except in Azare where *An. gambiae s.s* was only found alone. With regards to the indoor resting density of these sibling species of the *An. gambiae* complex in this study, it was found that higher population of *An. gambiae* s.s were recorded indoors than *An. arabiensis* which were more recorded outdoors.

Keywords: katagum area, bauchi-nigeria, *Anopheles gambiae* s.L; sibling species, indoor resting density

Introduction
Malaria, a mosquito-borne disease is one of the major health problems in Nigeria in particular and in other part of Sub-Saharan Africa. Statistics show that malaria accounts for 660,000 deaths worldwide. Every year, more than 200 million cases occur [1]. According to the latest world malaria report released in November, 2018, there were 219 million cases of malaria with estimated 435, 000 deaths in 2017 [2]. However, this report indicated that 92% of the world malaria cases with 93% of malaria deaths were recorded in Africa. In Nigeria, up to 60% of outpatient attendance in health facilities is due to malaria and 30% of all hospital admissions. It is estimated that malaria is responsible for 25% infant mortality, 30% childhood mortality and is associated with 11% maternal deaths. The economic burden of this disease in Nigeria is estimated to be N 132 billion lost annually in terms of treatment costs, prevention, loss of man hours etc. [3]. Most malaria deaths occur at home hence are not reported [4]. The disease can be attributed almost entirely to the mosquitoes *Anopheles gambiae*, *An. arabiensis* and *An. funestus*, three of the most efficient malaria vectors in the world. All live almost exclusively in close association with humans and feed on blood, primarily from humans [5].
Nigeria had witnessed a number of human activities in the last three decades which include agricultural development, construction of dams for irrigation and domestic consumption, construction of human habitation and deforestation. All these anthropogenic activities have the potential to provide breeding grounds for mosquitoes and thus expand the range of mosquitoes in all geographical ranges of Nigeria [6].
Accurate and precise identification of mosquito species and their possible sibling species is vital in planning vector strategies.

Since the sibling species cannot be distinguished by their external morphological features, molecular taxonomy is required to obtain reliable information about the identity of the vector species [7]. If meaningful control strategies are to be formulated against the malaria vectors in the Sahel, studies to confirm the predominant sibling species of *An. gambiae* sensu lato must be carried out.

In sub-Sahara Africa, the main malaria vectors that play major roles in transmission of the disease belongs to the group of the *Anopheles gambiae* Giles and *An. funestus* Giles. Although other (secondary) vectors such as *An. moucheti* and *An. nili* have been reported in some parts of Africa [8]. These groups comprise of diverse species whose distribution and composition vary from one geographical location to another being greatly influenced by the prevailing climatic conditions [9]. The *An. gambiae* complex comprises of seven morphologically indistinguishable species while *An. funestus* group consists of about nine morphologically identical species especially at the adult stage [10-12]. Identification of the members within the same morphologically identical taxon to species level is an essential component of epidemiological study of malaria. Since this will help to incriminate the responsible vector species as an important step in formulating strategic control programme.

However, cryptic within the taxon are difficult to identify using conventional morphological characters because of the morphological overlap between them. The need to avoid misidentification between the different sibling species of anopheline complexes for proper clarification of their epidemiological roles in disease transmission has led to the development of molecular assays for species identification [13-16]. The present study is aimed at using molecular assays to identify the different sibling species of *Anopheles gambiae* s.l., their abundance and distributions within the study area.

Materials and Methods

Study area

The study was carried out in Katagum area of Bauchi state. The area comprises of seven Local governments which includes Katagum, Giade, Itas/Gadau. Shira, Jama'are, Gamawa, and Zaki local governments, the area is located at about 198 km north of Bauchi town (i.e. the capital of Bauchi state) and covers about 10,203 km^2 (area) and a population of 1,503, 164 people at co-ordinates of 22^0 45N, 12^0 46E. Katagum area has a temperature of $30 – 35^0$ in the dry season and $26 – 28^0$ in the cold days (i.e. Nov – Jan.) while it is fluctuating during the rainy days. The area is surrounded by *Acacia nigrescens, Azadirachta indica* and many other trees. River flows downstream in many parts of the area thereby creating a wide wetland with a profusion of suitable breeding sites for mosquitoes [17].

Sample collection

Indoor resting adult mosquitoes collections were carried out every month during the study period (June – Dec, 2016) in each of the collection sites. Eight bedrooms (One room/household) were selected randomly from each site.

Collection of mosquitoes by the use of non-residual pyrethrum (Pyrethrum Spray Collection) was employed using World Health Organization (WHO) standard procedure [18, 19]. All knocked down mosquitoes were collected into properly labeled petri-dishes and transported to laboratory for identification and preservation for molecular bioassay.

Mosquito species identification

Identification of mosquito was done using standard morphological keys [10, 11]. Molecular assay was carried out using specific polymerase chain reaction (PCR) [16] with minor modifications as detailed in Van Rensburg *et al*. [20] for the confirmatory identification of the sibling species within *Anopheles gambiae* complex.

Ethical consideration

Letter of introduction was obtained from the Department of Biological Sciences, Faculty of Science, Abubakar Tafawa Balewa University, Bauchi State, Nigeria seeking ethical clearance from local chiefs in the study areas.

Data analysis

The indoor resting density (IRD) of female Anopheles mosquitoes was calculated using WHO [19] formulae as follows: IRD as number of females collected per number of houses sampled multiplied by the number of nights.

Results and Discussions

Mosquito abundance and their distributions

A total of two hundred (200) females mosquitoes were collected from June to December, 2016. Out of these, 40 (20%) were Anophelines while 160 (80%) were Culicines (Table 1). The month of July recorded the highest number of mosquitoes with peak values of 17 and 62 for the *An. gambiae*s. L. and the culicine species respectively. Then there after the number of both species of mosquitoes started to decline. However, in December no mosquito species was recorded at all. The dynamics in mosquito population at the study area may be associated the variations in rainfall pattern. The mosquito population increased from the onset of rain mostly in May – July and reduce during heavy rain (August – October). This could be attributed to the increase in the frequencies of rain falls from August - September that might facilitate the washing away of the developing larvae from their breeding habitats thereby leading to the decline in the population of mosquitoes. This effect is more serious with regards to Anopheline mosquitoes that require shallow and temporary breeding habitats as earlier observed by Gordon and Lavoipierre [21] and much emphasized by Charles and Godfray [22]. On the other hand, from October – December, the reductions in the abundance of mosquitoes in the study was associated with the decrease in the number of breeding sites as a result of the general decrease in the amount of rainfall.

Table 1: Monthly abundance of different mosquito species in Katagum area, Bauchi state, Nigeria, June – December, 2016.

S/N	Collection Period	*An. gambaie* s. L.	Culicine species	Total
1.	June	05	15	20
2.	July	17	62	79
3.	August	08	32	40
4.	September	03	16	19

5.	October	04	25	29
6.	November	03	10	13
7.	December	00	00	00
	Total	40	160	200

Table 2, shows the distributions of the *An. gambiae*s. L. and the Culicine mosquitoes across the collection sites. In this table, it is evident that Culicine mosquitoes collected from the different sampling sites were more abundant than the Anophelines (*An. gambiae*s. L.). However, Zaki and Itas sampling sites recorded the highest numbers of *An. gambiae*s. L. then followed by Yana and Jamaare which recorded equal number of the *An. gambiae*s. L. No *An. gambiae*s. L. was recorded in Giade during the period of this study. Similarly, Yana, Zaki and Itas recorded the highest number of Culicine mosquitoes with values increasing from 25 to 39 accordingly. The high abundance of Culicine mosquitoes within all the sampling sites in this study could be attributed to the fact that all the study sites are semi- urban towns and it has been earlier reported that Culicine mosquitoes are associated with urbanization [23] where they breed in sewage systems, gutters, sinks, wells, ponds or in any waste water found in the urban or rural environments.

Table 2: Mosquito species and their abundance at different collection sites in Katagum area, Bauchi state, Nigeria, June – December, 2016.

S/N	Collection Site	*An. gambaie* s. L.	Culicine species	Total
1.	Azare	05	20	25
2.	Giade	00	09	09
3.	Yana	06	25	31
4.	Zaki	10	30	40
5.	Itas	11	39	50
6.	Gamawa	02	16	18
7.	Jamaare	06	21	27
	Total	40	160	200

Molecular identification of *Anopheles gambiae* complex species

Results from the molecular identification (PCR) of the *An. gambiae*s. L. collected from the study areas revealed that *An. gambiae*s. s. and *An. arabiensis* were the only two sibling species of the *An. Gambiae* complex were recorded during this period of study (Table 3). It was only in Giade sampling area that none of these sibling species of the *An. gambiae* complex was recorded. This results also, evidently showed that the *An. gambiae*s. s. and *An. arabiensis* occurred together except in Azare where *An. gambiae*s. s. was only found alone. The results of the preset study further supported the findings of the earlier researches on the ecology of the members of the *An. gambiae*s. L. Of the seven siblings of the gambiae complex, *An. gambiae*s. s. and *An. arabiensis* which are the most important vectors of malaria [24], despite the fact that these two species share a continent-wide distribution [9, 8], they occur sympatrically in almost all the sampling sites and exhibit strong associations with the traditional rural life style of many African communities [25-28].

Table 3: Composition of the different sibling species of *An. gambiae*s. L., their relative Abundance (%) and Indoor Resting Density per collection site in Katagum area, Bauchi State, Nigeria, June-December, 2016.

S/N	Collection Site	*An. gambiaes.s.*	*An. arabiensis*
1.	Azare	12.5 (0.021)*	00 (00)*
2.	Giade	00(00)	00 (00)
3.	Yana	10.0 (0.017)	5.0 (0.008)
4.	Zaki	15.0 (0.025)	10.0 (0.042
5.	Itas	20.0 (0.033)	7.5 (0.031)
6.	Gamawa	2.5 (0.004)	2.5 (0.004)
7.	Jamaare	10.0(0.017)	5.0 (0.008)

()* Figures in brackets represent Indoor Resting Density of *Anopheles* species

Feeding behaviour of *An. gambiae*s.s. and *An. arabiensis*

The results for the indoor resting densities of *An. gambiae*s.s. and *An. arabiensis* in this study (Tables 3 & 4), clearly revealled that these two sibling species of the gambiae complex mosquitoes differ with regards to their feeding behaviours. However, more *An. gambiae*s.s. were recorded indoors than *An. arabiensis*. This could lead to the conclusion that *An. gambiae*s.s. may feed more often indoors (Endophagic) and rest indoors (Endophilic) than *An. arabiensis* which was reported to have high ecophenotypic plasticity with some females that are endophagic and could remain endophilic for 1 or 2 days [29]. However, majority of females An. arabiensis readily bite outdoors and the engorged females enter house to rest [10].

Table 4: Monthly relative abundance (%) of the different sibling species of *An. gambiae*s. L., and Indoor Resting Density in Katagum area, Bauchi State, Nigeria, June-December, 2016

S/N	Collection Period	*An. Gambiaes. S.*	*An. Arabiensis*
1.	June	7.5 (0.031)*	5.0 (0.021)*
2.	July	27.5(0.046)	15.0 (0.025)
3.	August	10.0 (0.017)	10.0 (0.017)
4.	September	5.0 (0.008)	2.5 (0.004)
5.	October	10.0 (0.017)	00 (00)
6.	November	5.0 (0.008)	2.5 (0.004)
7.	December	00(00)	00 (00)

()* Figures in brackets represent Indoor Resting Density of *Anopheles* species

Conclusions

Sibling species within the Gambiae complex display important differences in their geographical distributions. It is therefore, very essential to understand their composition and abundance for the design, implementation and continued evaluation of the appropriate malaria interventions, especially as control programmes work toward transmission reduction and elimination. The results obtained from this study indicated very low numbers of the different sibling species of the *Anopheles gambiae* s. L. within the study area this

translate to low indoor resting densities of the sibling species. This may suggest little input to achieve malaria control within the study area. Vector control interventions such as use of insecticide treated bed nets, well screened houses, and larval source managements can be of good help to control malaria transmission in this area of study.

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