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Distribution of Members of the *Anopheles Gambiae s.l.* In Oyo State, South West Nigeria

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Background: Members of the *Anopheles gambiae s.l.* remain the most efficient vectors of malaria parasite in Africa. However, for timely and effective vector control activities, the distribution of these important vectors in local communities is crucial. We therefore determine the distribution of the members of *Anopheles gambiae s.l.* in Oyo State, Nigeria

Methods: Larval stages of *Anopheles* mosquitoes were collected from identified mosquito breeding sites in six localities (Oluyole, Eruwa, Oyo, Ojoo, Bodija, and Ogbomoso) in Oyo State and reared to adults. Three to five days old adult emergence were identified morphologically using standard methods. A total of 100 mosquitoes were selected from each of localities for molecular analysis. DNA were extracted and Polymerase Chain Reaction (PCR-ID) followed by restriction endonucleases digestion was used for molecular identification.

Results: A total of 58 larval breeding sites were sampled out of which 12 (20.7%) had Anophelines only, 21 (36.2%) contained Culicines only and the remaining 25 (43.1%) had both Anophelines and Culicines. The mosquitoes were mostly found in footprints, followed by tire tracks, pools, puddle and ponds. The habitat type distribution for Anopheline and Culicines were not different ($\chi^2=5.25$, $DF=5$, $P>0.01$). A total of 1,725 Anophelines emerged from the collection out of which, 823 were females. All the female samples were morphologically identified as members of the *Anopheles gambiae s.l.*. A total of 600 (72.9%) of the female Anopheline population was subjected to PCR. PCR-ID showed that the mosquito populations contained higher numbers of *Anopheles arabiensis* (58%) than *Anopheles gambiae s.s.* (42%). Enzyme digest indicate that samples from Oluyole, Iwo road and Bodija were mainly the M form (now called *An. coluzzii*), while both M (*An. coluzzii*) and S (*An. gambiae*) form occur in sympatry in Oyo town and Eruwa.

Conclusion: This study presents information on the distribution of *Anopheles arabiensis*, *Anopheles coluzzii* and *Anopheles gambiae* in Oyo State. This has implication on the vector control activities in the State as members of these *Anopheles* mosquitoes exhibit varying feeding behaviours, transmission pattern and resistance profiles. Such information is useful in planning vector control activities for the State

Keywords: Distribution, *Anopheles gambiae s.l.*, *Anopheles coluzzii*, *Anopheles arabiensis*, *Anopheles gambiae*

1.0 INTRODUCTION

Malaria remain a serious public health disease in Nigeria accounting for 60% of outpatients visits and up to 30% of hospitalizations among children under five years of age [1]. Up to 85% of Nigerians live in areas supporting mesoendemic malaria transmission, 15% lived under conditions of hyper- holoendemicity while areas within Abuja, Adamawa and Borno states support hypoendemicity [2]. With an estimated population of 160 million and zonal variations ranging from 27.6% in the South-east to 50.3% in the South-west, the country has a large population at risk. The dominant species of malaria parasite in Nigeria is *Plasmodium falciparum* which accounts for up to 95% of the malaria cases and the most pathogenic of the five human malaria parasites [2]. *Plasmodium falciparum* have also been found to occur with other plasmodium species like *P. malariae* and *P. ovale* in Nigeria [3].

The transmission of malaria parasite depends largely on the availability of competent mosquito vectors [4]. In Nigeria, the primary malaria vectors include species in the *Anopheles gambiae* complex (including *An. gambiae*, *An. coluzzii* and *An. arabiensis*) and *Anopheles funestus* group. These species are widely distributed across the country, from the mangrove and coastal areas of the South to the Sahel savannah of the North. *An. gambiae* s.s., *An. arabiensis*, and *An. coluzzii* typically breeds in sunlit, shallow and temporary fresh water bodies such as ground depressions, puddles, pools and hoof prints. *An. funestus* breeds in permanent or semi-permanent body of fresh water with emergent vegetation which sustains malaria transmission during the dry season [2].

Anopheles coluzzii and *An. gambiae* are highly anthropophilic and endophilic with sporozoite rates ranging from 0.2-11.8%. *Anopheles arabiensis* and *An. funestus* are zoophilic and anthropophilic, with sporozoite rate of 0-4.8% and 1.2-2.3% respectively [2]. In most cases, sporozoite infection in the wet season are maintained by *An. gambiae* s.s. and *An. coluzzii* while *An. arabiensis* maintains transmission in the dry season [5]. Transmission indices show that the entomological inoculation rates (EIR) range from 18-145 infective bites per year for *An. coluzzii* and *An. gambiae* s.s. in the North central and 12-54 infective bites per year for *An. funestus* in the South west. The EIR estimated for *An. arabiensis* ranged between 0-12.4 [2].

One of the principal strategies for preventing and controlling malaria transmission vis-à-vis reduction in mosquito population is vector control [2]. Therefore, mapping the distribution of vectors and understanding

their behaviour is essential in planning vector control activities. However, there is paucity of data on the distribution of the major malaria vectors in Nigeria [2]. It is therefore important that reports are made available on the distribution of sibling species in space and time to inform specific vector control strategies against the malaria vectors. This study therefore examine the distribution of the major malaria vectors in Nigeria.

2.0 METHODOLOGY

2.1 Study area

The study was carried out in Oyo State, Southwest, Nigeria. Oyo state lies between longitude 8°00'N and latitude 4°00'E and has a total land mass of 28,454 km² (10,986.2 sq mi). It is bounded partly by Ogun State in the South and Kwara State in the North. To the West, it is bounded partly by Ogun State and partly by the Republic of Benin, while in the East it is bounded by Osun. The topography of the State is one of the gentle rolling lowland in the south, rising to a plateau 40 metres and above in the North. The vegetation pattern of the State is that of rain forest in the South and guinea savannah to the North. Thick forest in the South gives way to grassland interspersed with trees in the north. Agriculture is the main occupation as the climate in the State favors the cultivation of crops like maize, yam, cassava, millet, rice, plantains, cocoa, palm produce, cashew [6].

Figure 1: Map of Oyo State showing the collection points

2.2 Mosquito Larval Collection and Rearing

Anopheles mosquitoes were identified from six localities (Oluyole, Eruwa, Oyo, Ojoo, Bodija and Ogbomoso) in Oyo state (Fig. 1). Identified breeding sites were mapped with Garmin eTrex® GPS 10 personal navigator. Following a standard protocol [7], larval samples were collected by lowering white dippers gently into identified breeding sites at an angle of 45° to the surface until one side is just below the surface. These were moved along the breeding site, skimming the surface of the water with the dipper and raised out of the water, making sure that the water containing the larvae and pupa does not spill. The larval and pupal samples were transferred into collection bottles, properly labelled per locality and taken to the Molecular Entomology and Vector Control Research laboratory of the Nigerian Institute of Medical Research, Yaba, Lagos. Upon arrival at the insectary, larval samples, kept in larval bowls, were maintained under standard insectary conditions (25-28°C and ~70-80% humidity, with a 12hr day/night cycle) [8] and fed with yeast daily. Adult emergence were fed with 10% glucose solution (with cotton wool soaked in water containing 10% sugar solution and placed inside the cage).

2.3 Morphological Identification of Mosquito species

The adult emergence were identified morphologically with the aid of Gilles and Coetzee [9] identification guide. Anopheline mosquitoes were identified with their speckled legs, hind tarsus 4-5 entirely pale, abdominal segments without laterally projecting tufts of scales, 3 bands on the palps, 3rd main dark area (preapical dark spot) of vein 1 with pale interruption, sometimes fused with the preceding pale spot and scaling on abdomen very scanty often confined to 8th or rarely 7th terga belonged to the *An. gambiae* s.l.. Upon proper morphological identification, mosquitoes belonging to this complex were further identified using Polymerase Chain Reaction (PCR) technique.

2.4 Molecular Identification of Mosquito species

One hundred morphologically identified *An. gambiae* s.l. were selected from each locality for molecular identification. DNA extraction was conducted with the aid of a genomic DNA extraction kit prepGEM™ insect (ZyGEM Corporation Limited, New Zealand). DNA was also extracted from the positive controls available at the Molecular Entomology and Vector Control unit, Nigerian Institute of Medical Research, Lagos. 1µl of the DNA was used as template for the PCR. For the species identification of *An. gambiae* complex, a standard protocol was adopted

[10]. Master mix solution containing 2.5µl of 10x PCR reaction buffer, 2.5µl of dNTP, 1µl of MgCL solution, 1µl of *An. quadriannulatus* primer, 2µl each of the other primers (Universal, *An. gambiae* s.s., *An. arabiensis*, *An. merus/melas*), 4.9µl of sterile distilled water and 0.2µl Taq polymerase enzyme. The PCR conditions include 94°C for 2min (denaturation phase), 30 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30 seconds (Hybridization and Extension phase), and a final extension phase of 72°C for 5min. 12.5µl reaction volume of the product was electrophoresed in 2.5% SEAKEM® agarose gel containing ethidium bromide and photographed under ultraviolet light illuminator. *Anopheles gambiae* s.s. present in the population were further identified as *An. coluzzii* and *An. gambiae* using restriction endonuclease. 12.5µl of the PCR product was digested by adding, 2µl of *Heamophilus haemolyticus* (HhaI) restriction enzyme, 18µl of distilled water, and 2µl of 10x Buffer Tango™. Reaction mixture was incubated for 4 hours at 37°C as previously described [11]. The reaction was stopped by adding 2µl of 0.5M EDTA. The entire product was electrophoresed in 2.5% SEAKEM® agarose gel containing ethidium bromide and photographed under ultraviolet light illuminator.

2.5 Data Analysis

Data was analysed with SPSS version 23.0. Habitat preference of Anophelines and Culicines were subjected to chi-square distribution. Percentage occurrence of Anopheline only, Culicine only and presence of Anopheline and Culicine was also calculated. For species identification, percentage occurrence of each species was calculated.

3.0 RESULTS

3.1 Larval Habitat Type and Habitat Preference

The A total of 58 mosquito larval habitats were sampled (Table 1) out of which 12 breeding sites contained anopheline only, 25 contained culicines only while the remaining 21 had a mixture of culicines and anophelines. Larval habitat preference was heterogeneous across the sites. Anopheline mosquitoes were found in puddles and tire tracks, while culicines were found in footprints, pools, puddle, and tire tracks. Both anophelines and culicines were found in footprints, ponds, and puddles (Table 1). The breeding site distribution for habitats with anopheline only or culicine only larvae was not different (Table 1; $\chi^2 = 5.25$, degree of freedom [df] = 5, $P > 0.01$).

Table 1. Distribution and abundance of Anophe-line and Culicine mosquito larvae in 58 aquatic habitats.

Larval habitat type	Anophe-line larva	Culicine larva	Anopheline and Culicine larva	Total
Footprints	0	17	9	26
Ponds	0	2	0	2
Pool	0	0	11	11
Puddle	3	2	1	6
Tire tracks	9	0	4	13
Tanks	0	0	0	0
Total	12 (20.7%)	21 (36.2%)	25 (43.1%)	58

3.2 Abundance and Distribution of Members of the *Anopheles gambiae* s.l. Across the Six Sites

A total of 600 *An. gambiae* s.l. (100 per site) were further identified using PCR (Table 1, Figure 2, Plate 1). The remaining specimens could not be identified due to lack of PCR products. In total, *An. gambiae* s.s. and *An. arabiensis* were the only members of the *An. gambiae* complex found across all sites. On the average, *An. arabiensis* (58%) was more abundant than *An. gambiae* s.s. (42%). There were more *An. gambiae* s.s. found in the population from Oluyole (87% *An. gambiae* s.s. vs. 13% *An. arabiensis*), Oyo town (73% *An. gambiae* s.s. vs. 27% *An. arabiensis*) and Eruwa (53% *An. gambiae* s.s. vs. 47% *An. arabiensis*) (Table 2), while the population from Iwo road and Bodija contained more *An. arabiensis* than *An. gambiae* s.s. However, all the mosquitoes analysed from Ojoo samples were *An. arabiensis* (Table 2).

Plate 1: Polymerase Chain Reaction Gel electrophoresis image of some of the field collected samples.

Lane 1 – Ladder/Marker; Lane 2- negative control; Lane 3-6 – positive controls (*An. gambiae* s.s., *An. arabiensis*, *An. melas/merus*, *An. quadriannulatus* respectively); Lane 7-10, 12-17, 19, 20 – field samples (*An. gambiae* s.s.); Lane 11 and 18 – field samples (*An. arabiensis*).

Table 2. Distribution of members of *An. gambiae* complex and *An. gambiae* s.s. molecular forms in the studied localities

State	Localities	No. examined	PCR – Species Identification		PCR - Form	
			% <i>An. gambiae</i> s.s.	% <i>An. arabiensis</i>	% “S”	% “M”
Oyo	Oluyole	100	87%	13%	-	100%
	Iwo road	100	23%	77%	-	100%
	Bodija	100	17%	83%	-	100%
	Ojoo	100	-	100%	-	-
	Oyo town	100	73%	27%	5%	95%
	Eruwa	100	53%	47%	50%	50%
	Total	600	42%	58%		

3.3 Abundance and Distribution of Members of the *Anopheles gambiae* s.s. (*An. gambiae* and *An. coluzzii*) across the six sites

Only the *An. gambiae* s.s. in the populations were analysed for PCR-form using restriction digest (Plate 2). Oluyole, Iwo road and Bodija population contained purely *An. coluzzii* (M molecular form) while the population from Oyo town and Eruwa had sympatric occurrence of *An. gambiae* and *An. coluzzii* (Table 2, Figure 3, Plate 2). Oyo town contained more *An. coluzzii* than *An. gambiae* (95% *An. coluzzii* vs. 5% *An. gambiae*) but there was equal abundance of both species in Eruwa (50% *An. coluzzii* vs. 50% *An. gambiae*) (Table 2). However, samples from Ojoo were not analysed for these species because they were mainly *An. arabiensis*.

Figure 2. Abundance and Distribution of the members of *Anopheles gambiae* complex identified from the six localities sampled in Oyo State

Plate 2: Gel electro gram of some of the Molecular digest (M and S form) of PCR products of *An. gambiae* S.S.

Lane 1 and 18 –Ladder/Marker; Lane 2- negative control; Lane 3- positive control (*An. gambiae* s.s.); Lane 4- positive control M form (*An. coluzzii*); Lane 5-9, 11, 13-17 – Field samples M form (*An. coluzzii*); Lane 10 and 12 – field samples (*An. gambiae* s.s.)

Figure 3. Abundance and Distribution of the members of *Anopheles gambiae* s.s. (*Anopheles coluzzii* “M form” and *Anopheles gambiae* “S form) identified from the localities sampled in Oyo State

4.0 DISCUSSION

Although, this study did not examine the influence of environmental variables on larval site preference, it seem Anophelines and Culicines larva primarily breed and co-exist freely in most of the habitats examined as the study did not detect any statistically significant associations between breeding site preference and mosquito occurrence and abundance. This study provide data on the distribution of Anopheline mosquitoes in some parts of Oyo State. Larval habitat preference was highly heterogenous for Anophelines and Culicines across the sites sampled. However, this study did not identify the possible environmental variables that determine anopheline occurrence and abundance in relation to larval habitats. This is because the heterogeneity in *An. gambiae* s.l. species composition has been reported to be affected either by many variables, each of which has a small effect, or by other

important variables that have not yet been measured under field conditions [12]. This is also consistent with the results of Robert et al [13] who found that the occurrence and abundance of one of the major malaria vectors, *An. arabiensis* larvae in permanent habitats in Dakar, Senegal, are determined by many physicochemical and biological variables.

Malaria vector control either by Indoor Residual Spray (IRS), Long Lasting Insecticide Treated Nets (LLINs) or genetic control strategies requires accurate mosquito identification and information on the behaviour of vector species which informs the choice of control strategies to deploy. Although the distribution of members of the *An. gambiae* is well documented in Africa [9], much of the work is still not well documented in Nigeria. Most of the work conducted in Nigeria focused on the dynamics and insecticide resistance status of the malaria vectors [5, 14-19]. However, there has been less emphasis on the distribution of these important vectors especially in the studied localities [14, 20, and 21].

There has been no report showing the distribution of these malaria vectors in Oyo State. Data available are that of spot checks involving samples exposed to insecticides [14, 22]. Our map shows the sympatric occurrence of *An. gambiae* s.s. and *An. arabiensis* across all sites examined with the absence of other species of Anopheles. The paucity of data from these areas affirms the presence of both species of Anopheles as detected in this study. This would serve as a guide for vector control activities in these localities. The dominance of the M molecular form (now *An. coluzzii*) in the *Anopheles gambiae* s.s. populations reported from Oluyole, Iwo road, Bodija and Oyo town is not surprising and may be due to the greater adaptive features of the M form as compared with the S (now also called *An. gambiae*) [23,24]. Both *An. coluzzii* and *An. gambiae* occur in sympatry at Oyo town and Eruwa. The presence of these molecular forms in our study confirms the earlier reports by Awolola [14] on the presence of the two molecular forms in certain parts of South west Nigeria. However, our report is the first to present the presence of *An. coluzzii* and *An. gambiae* occurring in sympatry from Oyo state populations.

Our map suggests the absence of *An. gambiae* s.s. in Ojoo. This is in contract to an earlier report [22]. The disparity in the data may be attributed to the method of collection and the low number of Anopheles mosquitoes tested by PCR in earlier studies from this locality. Up to date, there is no spatially- continuous map of Anopheles mosquitoes from the selected localities. Our introduced approach, however, yielded more finely resolved *An. gambiae* s.l. distribution in Oyo state.

These data provide valuable information for selective and targeted malaria vector control in some parts of Oyo State. The maps may also be useful for planning future implementation of malaria control by genetically manipulated mosquitoes. In addition, temporal distribution maps of the molecular forms would be useful especially in areas where they occur in sympatry [24,25].

This study represents a continuous distribution map of the members of *An. gambiae* s.s. in Oyo State, Nigeria. The study provide valuable information for selective vector control in the state and may serve as a decision support tool for future malaria control strategies including genetically manipulated mosquitoes in the areas.

Conflict of interest

Authors declare that there is no conflict of interest

Authors' Contributions

AOA conceived and designed the study, collected data, contributed to data analysis and revised the manuscript. **KOKP** performed study design, drafted the manuscript and contributed data analysis. **AKO** performed data analysis. **STA** conceived and performed study design. All authors approved the final manuscript.

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