Species composition of the *Anopheles gambiae* complex across eco-vegetational zones in Bayelsa State, Niger Delta region, Nigeria

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ABSTRACT

Background & objectives: Correct vector identification is an important task in the planning and implementation of malaria vector control programmes. This study was designed to provide baseline information on the species composition and distribution of members of the *Anopheles gambiae* complex in three eco-vegetational zones in Bayelsa state, Nigeria.

Methods: Adult mosquitoes were collected by pyrethrum spray catch (PSC) in randomly selected houses during September 2009–August 2010. *Anopheles* mosquitoes were identified using standard morphological keys. Mosquitoes identified as *An. gambiae s.l.* were used for species specific PCR-assays.

Results: Out of 203 *Anopheles gambiae s.l.* successfully amplified, 180 (88.7%) were *Anopheles gambiae s.s.*, 14 (6.9%) were *An. melas* and 9 (4.4%) were *An. arabiensis.* The variation in the sibling species composition of *An. gambiae s.l.* was not significant (p > 0.05). *Anopheles gambiae s.s.* was predominant in all the collections with three sibling species occurring in all the eco-vegetational zones.

Interpretation & conclusion: The observation of *An. melas* in the fresh water swamp forest of Yenagoa is of importance in malaria epidemiology. These findings are of importance in the planning and implementation of malaria vector control strategy in the three eco-vegetational zones of Bayelsa state.

Key words Anopheles gambiae s.l.; Bayelsa state; distribution; eco-vegetation; Nigeria; PCR

INTRODUCTION

The Anopheles gambiae sensu lato (s.l.) is the most important vector of malaria in Africa¹ and comprises of seven morphologically indistinguishable sibling species². The most important members of the complex involved in malaria transmission are *An. gambiae s.s.* and *An. arabiensis. An. quadriannulatus, An. melas, An. merus* and *An. bwamba* are of minor importance^{3–7}. These species vary in relative frequency and distribution from one geographical location to another; distribution is influenced by prevailing climatic conditions^{8,9}. In Nigeria, higher populations of *An. arabiensis* were recorded in the arid northeast region¹⁰, while higher populations of *An. gambiae s.s.* were collected in the southern tropical rainforest^{8,11}.

The identification to species level of morphologically identical taxa within the complex is an essential component of the epidemiological study of malaria and the subsequent formulation of control strategies in malaria endemic areas^{12,13}.

There is paucity of information on the composition and distribution of members of the *An. gambiae* complex in Bayelsa state, Niger Delta region, Nigeria. This study utilized polymerase chain reaction (PCR) assays to identify the sibling species of the major vector of malaria, *An. gambiae* complex in different eco-vegetational zones of the state.

MATERIAL & METHODS

Study area

The study was conducted at seven localities in Bayelsa state (5° 22' E, 6° 45' E and 40° 14' N, 5° 23' N). Bayelsa is located within the lower Delta plain formed during the Holocene of the Quaternary period by the accumulation of sedimentary deposits¹⁴. The vegetation comprises three eco-vegetational zones: fresh water swamp forest, brackish water forest and mangrove forest. The average annual rainfall varies from 3000–4000 mm². The topography of the study area is characterized by a maze of creeks and swamps, criss-crossing the low-lying plain. The study towns/villages were: Yenagoa, (4º 53' N and 5º 17' E), Sagbama (5°09' N and 6°14' E), Kaiama (5°09' N and 6° 14' E) in the fresh water swamp forest; Ogbia (4°36' N and 6º 14' E), Oporoma (4º 15' N and 6º 14' E) and Ekeremor (5° 02' N and 5° 48' E) in the brackish water swamp forest and Nembe (4° 27'N and 6° 26' E) in the

mangrove forest. Houses were of traditional architecture with mud walls and thatched roof, while few were built with blocks and corrugated iron sheets. The major occupation of the people is fishing, farming and petty trading.

Mosquito sampling

Consent was obtained from village and household heads before the commencement of the study. Adult mosquitoes were collected twice in each quarter during September 2009 to August 2010. The pyrethrum spray catch (PSC) method for mosquito collection was used during 0600–0730 hrs^{15,16}. Selected rooms were those with at least one person overnight. Prior to spraying, the floors were covered with clean white sheets. Pyrethroid was sprayed with doors closed. There was a 15 min interval before the removal of the sheets. These were inspected for anophelines and transferred with forceps into labelled petri dishes. Subsequently, taken to the laboratory for morphological identification with keys¹⁷.

PCR-identification of members of An. gambiae complex

The species-specific PCR assays¹⁸ were undertaken at the Nigerian Institute of Medical Research, Yaba, Lagos. The deoxyribonucleic acid (DNA) of each mosquito sample identified as An. gambiae s.l. was extracted from legs or wings after Collins et al¹⁹. One µl of extracted DNA from each mosquito sample was added to 12.5 µl aliquot of master mix (1.25 µl dNTP, 0.5M MgCl₂, 4.9 µl H₂O, 0.1 Taq, +1.0 of each primer). The DNA amplification of An. gambiae complex in a thermal cycler (Primus 96 PCR-system MWG Genomic Technology) consisted of an initial denaturation step at 94°C for 1 min; 30 cycles each of 30 sec denaturation at 94°C, 30 sec annealing at 50°C and 30 sec extension at 72°C. The final extension was at 72°C for 10 min. PCR products were separated on 2% agarose gel (2 g of Agarose in 100 µl TAE buffer), stained with ethidium bromide and viewed on ultraviolet transilluminator.

Data analyses

Fisher's exact and chi-square tests were used to test for the variations in *An. gambiae* sibling species abundance.

RESULTS

A total of 350 mosquitoes identified as female An. gambiae s.l. were PCR-assayed; 203 (58%) were successfully amplified while 147 (42%) failed to amplify. The amplified 203 consisted of 180 (88.7%) An. gambiae s.s., 14 (6.9%) An. melas and 9 (4.4%) An. arabiensis. Variation in sibling species composition was not statistically significant (F = 2.183, p > 0.05) (Table 1). When the An. gambiae sibling species were pooled across eco-vegetational zones, the proportions of An. melas and An. arabiensis were more abundant in the mangrove forest than in the other eco-vegetational zones, but An. gambiae s.s. was significantly more abundant in the three eco-vegetational zones ($\chi^2 = 55.7$, df = 2, *p* <0.05) (Table 2). Anopheles melas was also recorded at one site in the fresh water swamp forest of Yenagoa and seasonal variations in the abundance of the An. gambiae sibling species were observed. Anopheles gambiae s.s. (88.9%) and An. melas (7.4%) were predominant during the wet season. The proportion of An. arabiensis (14.3%) increased in the dry season. However, seasonal variations in An. gambiae sibling species were not significant ($\chi^2 = 5.51$, df = 2, p > 0.05) (Table 2).

DISCUSSION

The record of three members (*An. gambiae s.s., An. arabiensis* and *An. melas*) of the gambiae complex in the three eco-vegetational zones of Bayelsa state was the first report of the occurrence of these sibling species of *An. gambiae s.l.* in the heartland of the Niger Delta region,

Table 1. Species composition of the An. gambiae complex across the study locations

Location	Number (%) of identified An. gambiae sibling species						
	An. gambiae s.s.	An. arabiensis	An. melas	Total amplified	Not amplified		
Yenagoa	32 (17.8)	1 (11.1)	1 (7.1)	34	2		
Sagbama	21 (11.7)	_	_	21	18		
Kaiama	21 (11.7)	_	_	21	19		
Ogbia	26 (14.4)	1 (11.1)	1 (7.1)	28	34		
Oporoma	25 (13.9)	_	_	25	15		
Ekeremor	21 (11.7)	3 (33.3)	_	24	19		
Nembe	34 (18.9)	4 (44.4)	12 (85.7)	50	40		
Total	180 (88.7)	9 (4.4)	14 (6.9)	203	147		

Zones/Seasons	Numbers identified	Species composition No. (%)		
		An. gambiae s.s.	An. arabiensis	An. melas
Eco-vegetational zones				
Fresh water swamp forest	76	74 (97.4)	1 (1.3)	1 (1.3)
Brackish swamp forest	77	72 (93.5)	4 (5.2)	1 (1.3)
Mangrove coastal forest	50	34 (68)	4 (8)	12 (24)
Total	203	180 (88.7)	9 (4.4)	14 (6.9)
Seasons				
Dry	14	12 (85.7)	2 (14.3)	0 (0)

168 (88.9)

180 (88.7)

Table 2. Species composition of An. gambiae complex in three eco-vegetational zones and during different seasons

Nigeria. Anopheles gambiae s.s. and An. arabiensis were collected at Sapele, located on the periphery of the Niger Delta²⁰. The occurrence of these sibling species had also been reported in the coastal region of Lagos, southwestern Nigeria^{16,21}. The failed PCR amplification recorded in the samples identified as An. gambiae s.l. was probably due to low DNA extraction and possible DNA degradation associated with storage or freezing and thawing of DNA; reports of similar problems are documented²².

189

203

The higher abundance and widespread distribution of An. gambiae s.s. across the three eco-vegetational zones was similar to other reports that highlighted widespread occurrence of An. gambiae s.s., the major malaria vector in Nigeria^{8,22,23}. The lower proportions of An. arabiensis reported in this study, contrasted with the moderately high proportion of An. arabiensis (44.6-55.4%) at Igbo-Ora in a Guinea savannah ecological zone²⁴ and An. arabiensis constituted 95% of the An. gambiae population in the Sahel savannah region¹⁰. Anopheles arabiensis had always been considered a species of arid areas^{10,25,26}. The low numbers of An. arabiensis in the Niger Delta are, therefore, not surprising. However, they indicate the progressive adaptation of An. arabiensis to more humid areas. The observed southward advance of An. arabiensis highlights the limitation in the exclusive reliance on climate and distributional data or the use of spatial imaging to predict species occurrence^{9,25}.

Relative abundance of members of the An. gambiae complex was also observed to be affected by seasons and the eco-vegetational type. Similar observations on the effect of seasons on the abundance of members of the An. gambiae had been reported²⁷. The predominance of An. arabiensis in the brackish swamp forest and An. melas in mangrove/coastal forest was similar to earlier results²³. The occurrence of An. melas, a brackish species, usually associated with mangrove forests, at a location in the fresh water swamp forest of Yenagoa was surprising. This species had been reported in the fresh water swamp forest of northeastern Tanzania²⁸. The propensity for inland distribution and adaptations of An. melas to breed in fresh water in southern Africa had been documented⁴. However, this was the first report of the occurrence of An. melas in an inland fresh water area in Nigeria.

7 (3.7)

9. (4.4)

14 (7.4)

14 (6.9)

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Wet

Total

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