



MORPHOLOGICAL IDENTIFICATION OF MALARIA VECTORS WITHIN ANOPHELES SPECIES IN PARTS OF KANO STATE, NIGERIA

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ABSTRACT

This study was carried out between May and October, 2010 to morphologically identify Anopheles species in parts of Kano State. A total of 2374 Anopheles were collected and identified. 1782 (75.07%) were collected from Nassarawa Local Government Area while 592 (24.93%) were collected from Tarauni Local Government Area. Using Anopheles characters of Gilles and Coetzee (1987) under zeiss light microscope, 587 (24.75%) were Anopheles funestus, 1535 (64.65%) were gambiae s.l. and 252 (10.60%) were An. maculipalpis. Nassarawa Local Government had the higher of Anopheles identified. Anopheles gambiae s.l. ranked the highest among other species. Further molecular identification of sub-species complex of An. gambiae s.l. and An. funestus is strongly recommended in the area.

Keywords: Identification, malaria, vectors, anopheles, species.

INTRODUCTION

Malaria remains a leading cause of morbidity and mortality worldwide with an estimated 500 million cases and 2.5 million deaths annually (Stauffer *et al.*, 2003). *Anopheles gambiae s.l.* and *An. funestus* transmit the *Plasmodium* parasites in sub-saharan Africa among the human population. Determination of risk of malaria transmission requires quick and accurate methods of identification of *Anopheles* mosquitoes especially when targeting vector control (Maxwell, *et al.*, 2003).

Anopheles mosquito transmits malaria. The most important vectors of malaria are members of *Anopheles gambiae s.l.* (complex), a group of morphologically identical yet genetically and behaviorally distinct species that differ markedly in their ability to transmit the diseases (Coluzzi, 1978). Members of the species complex include *Anopheles gambiae s.s.*, *An. Arabiensis*, *An. merus*, *An. melas*, *An. bwambe*, and *An. quadriannulatus* (Coetzee *et al.*, 2000).

Anopheles mosquitoes breed in areas with water bodies such as ponds, rivers, surface water, waste-waters, well, etc (Service, 1980). Moreover, these areas are suitable for the growth and development of various strains of mosquitoes as ponds, wells and surface water-bodies of different sizes are available during rainy season, May to October and supplemented waste-water throughout the year which could also serve as breeding sites. Molineaux and Grammicia (1980) concluded that differences in *Anopheles* species cause the failure of insecticide application and mass drug administration in suppressing malaria transmission. The present *Anopheles* status of the study areas is not known. Therefore, the aim of this study is to identify various *Anopheles* species in the area for effective vector control.

MATERIALS AND METHODS

Study Area

The study was carried out in Nassarawa and Tarauni Local government Areas of Kano State, Nigeria between May and October, 2010. The area is situated at latitude 12°35' N and longitude 8°30' E. It is about 840km from the edge of the Sahara desert and 475.45 meters above sea level. It also falls mostly within the Sudan savannah zone, it is semi arid region. The minimum and maximum temperature range between 15.86°C and 33°C and falls as low as 10°C during harmattan season between December and February. Rainfall ranges from 500mm to 1200mm and starts from May and ends in October, while dry season starts in November and ends in April (Wikipedia, 2010). More water bodies that harbor survival of various strains of *Anopheles* are observed in the area.

Sample Collection

Indoor Collection

Indoor resting adult mosquitoes were collected according to (Molineaux *et al.*, 1980). The collection was systematic; the selected houses were visited between 6 am and 10 am every other day for ten days. A sheet of large white cloth (4m x 3m) was spread on the floor in a room for easy recognition of mosquitoes and this was followed by spray of pyrethrum (Pyrethrin). After 15 minutes, the mosquitoes that fall on the sheet of white cloth were collected and stored separately in eppendorf tubes containing anhydrous calcium sulphate (CaSO₄) as drying agent until required (Molineaux, 1980). Twenty four houses were visited in this study.

Collection and Rearing of Larvae

Weekly surveys were conducted to establish the availability of stagnant water, habitat characteristics and larval densities from August 2010 and to October, 2010 in all habitats identified.

Larval sampling was done using the standard dipping method with a 350ml mosquito scoop (Bioquip, Gardena, CA, USA) as described by Service (1993). The number of dips taken from each habitat was dependent on the perimeter of the larval habitat. Larval habitats were categorized depending on their size in perimeter was grouped into three classes as $\leq 10\text{m}$, 10-100m and $> 100\text{m}$, and a maximum of 10, 50 and 50 dips were taken from these habitats, respectively. The number of larvae per dip (total number of larvae/no of dips). The *Anopheline* larvae were sampled and the stage of larval development recorded as either 1st – 2nd instars (early), 3rd – 4th (late) instar or pupae. The larvae were immediately preserved in plastic jugs and taken to laboratory for rearing according to WHO (1975a and 1975b). They were kept at room temperature and fed with ground fish diet powder in Aquarium. The adults that emerged (within 1-4 days) were killed by anaesthetizing using drops of Acetyl acetate placed on large Whatman's filter paper above the adults' container. They were collected and stored separately in Eppendorf tubes prior to identification.

Morphological Identification of *Anopheles* Mosquitoes

Using morphological characters of Gilles and Coetzee (1987) under x 20 Zeiss light microscope. The identification focused dark spot at the upper margins of the wings which is common to all *Anopheles*. The palps are elongated and segmented into three. A pale spot on second dark area, a light spot between the two dark spots on vein 6 two dark spots on vein 6 and absence of fringes on vein 6 are features for *Anopheles funestus*. Speckles on the legs, third preapical dark area on vein 1 with a pale interruption and tarsi 1-4 with conspicuous pale bands are features for *Anopheles gambiae*. Vein 1 with 2 accessory sector pale spots, hind tarsi 4 and 5 entirely pale and legs are pale are features for *Anopheles maculipalpis*.

Data Analysis

The data obtained in the study were collated and analyzed with respect to *Anopheles species*

abundance in the study area. These were interpreted in percentage and presented in tables.

RESULTS

A total of 2374 *Anopheles* were identified of which 592 (24.93%) and 1782 (75.07%) were collected from Tarauni and Nassarawa Local Government Areas respectively. Males and females were 1127 (47.47%) and 1247 (52.53%) respectively, *Anopheles funestus* were 587 (24.75%), *An. gambiae s.l.* were 1535 (64.64%) and *An maculipalpis* were 252 (10.60%). The adults collected indoors from Tarauni were 382 (64.52%) while 210 (35.48%) were adults reared from larvae. Adults collected indoors from Nassarawa were 446 (25.02%) while 1336 (74.97%) were adults reared from larvae.

Table 1 shows that range of *Anopheles* species adults collected indoors from Tarauni local Government Area. In this 382 *Anopheles* species were identified of which 105 (27.49%) were *An. funestus*, 230 (20.60%) were *An. gambiae* and 47 (12.3%) were *An. maculipalpis*. 158(41.36%) were males while 224 (58.64%) were females. Table 2 shows the range of *Anopheles* species adults reared from larvae from Tarauni. There were 210 identified of which 65 (30.95%) were *An. funestus*, 124 (59.05%) were *An. gambiae* and 21 (10.00%) were *An. maculipalpis*. 68(32.38%) were males while 142(67.62%) were females.

Table 3 shows the range of *Anopheles* species adults collected indoors from Nassarawa Local government Area. 446 *Anopheles* were identified of which 140(31.3339%) were *An. funestus*, 206(46.19%) were *An.gambiae* and 100(22.42%) were *An. maculipalpis*, 218(48.88%) were males while 228(51.12%) were females. Table 4 shows the range of *Anopheles* species adult reared from larvae from Nassarawa. 1336 *Anopheles* were identified of which, 277 (20. 73%) were *An. funestus*, 975 (172.98%) were *An. gambiae* and (251.12%) were males while 653 (48.88%) were females.

Table 1: Distribution of *Anopheles* Species Adults Collected Indoors from Tarauni Local Government Area

<i>Anopheles species</i>	No. of males identified	No. of females identified	No. of overall (male and female)
<i>funestus</i>	50 (31.65%)	55 (24.55%)	105 (27.49%)
<i>gambiae s.l</i>	82 (51.90%)	148 (66.07%)	230 (60.20%)
<i>maculipalpis</i>	26. (16.45%)	21 (09.38%)	47 (12.31%)
Total	158 (41.36%)	224 (58.64%)	382

Table 2: Distribution of *Anopheles* Species Adults reared from larvae from Tarauni Local Government Area

<i>Anopheles species</i>	No. of males identified	No. of females identified	No. of overall (male and female)
<i>funestus</i>	28 (41.18%)	37 (26.15%)	65 (30.95%)
<i>gambiae s.l</i>	31 (45.59%)	93 (65.49%)	124 (59.05%)
<i>maculipalpis</i>	09 (13.23%)	12 (08.45%)	21 (10.00%)
Total	68 (32.38%)	142 (67.62%)	210

Table 3: Distribution of *Anopheles* Species Adults collected indoors from Nassarawa Local Government Area

<i>Anopheles species</i>	No. of males identified	No. of females identified	No. of overall (male and female)
<i>funestus</i>	72 (33.03%)	68 (29.83%)	140 (31.39%)
<i>gambiae s.l</i>	97 (44.50%)	109 (47.81%)	206 (46.19%)
<i>maculipalpis</i>	49 (22.47%)	51 (22.36%)	100 (22.42%)
Total	218 (48.88%)	228 (51.12%)	446

Table 4: Distribution of *Anopheles* Species Adults reared from larvae from Nassarawa Local Government Area

<i>Anopheles species</i>	No. of males identified	No. of females identified	No. of overall (male and female)
<i>funestus</i>	163 (23.86%)	114 (17.46%)	277 (20.73%)
<i>gambiae s.l</i>	472 (69.11%)	503 (77.03%)	975 (72.98%)
<i>maculipalpis</i>	48 (07.03%)	36 (05.51%)	84 (06.29%)
Total	683 (51.12%)	653 (48.88%)	133

DISCUSSION

The number of *Anopheles* mosquitoes identified 2374 is very high. Spraying insecticides can reduce vector infectivity by reducing the vector survival rate and increasing the length of the sporogonic cycle (Anonymous, 1991). For example, when indoor resting mosquitoes are forced to rest outside, where ambient temperature is suboptimal for parasite maturation and eventual vector survival (Lines *et al*; 1991). Nassarawa Local Government Area had the higher number of *Anopheles* 1782 (75.07%); this may be due to environmental management of breeding sites as reported by service (1980); as some of the environmental management practices to include reduction and or management of breeding sites by filling container receptacles, water storage Jars, village pot, tyres, canoes, abandoned cans. Breeding sites like ponds burrow pits, fresh and salt mashes can be drained or impoundments built, which could lead to permanent control.

Most of the *Anopheles* reported in this study were similarly found by Coluzzi, *et al.* (2002) in that the most important vector of malaria parasite in sub-saharan Africa is *An. gambiae s.l*. It exhibits extreme heterogeneity. This had tallied with this research whereby most of the *Anopheles* identified were *An. gambiae s.l*. 1535 (64.65%).

The result of morphological examination of adults from larvae has revealed the presence of

predominantly *An. gambiae s.l* (Table 2 and 4). This observation is important; as it reveals that *An. gambiae* and other species are breeding in the study area.

The study also revealed the abundance of *An. funestus* and *An. maculipalpis* in relatively low proportion in the adults indoor collections in Tarauni Local Government Area than in Nassarawa Local Government Area. This could be attributed to the vector survival parameters such as the abundance of breeding site among others.

Identification of *Anopheles* species like all other mosquitoes is changing where taxonomist are rather describing new species and subspecies or re-describing existing one. Many techniques used in the identification of *Anopheles* mosquitoes have been published including (Faran, 1981) and (Gillet, 1972).

CONCLUSION

The promising result of morphological identification of malaria vectors within *Anopheles* species in this study justified the presence of *An. funestus*, *An. maculipalpis* and *An. gambiae s.l*. in the study area.

RECOMMENDATION

Further molecular characterization of the *Anopheles* identified into *An. Arabiensis*, *An. gambiae s.s*, M and S forms is strongly recommended.

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