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Morphological Identification, Species Composition and Distribution of Mosquitoes in Kosti region, White Nile State, Central Sudan

Mai M. Nour1, Mariam Atta Elmannan Aboud2, Nahla Osman Mohamed Ali3*

1,2Department of Biology and Environmental Studies, El Neelain University, Khartoum. P.O. Box: 12702, SUDAN
3Department of Parasitology, University of Khartoum, P. O. Box 32, Shambat Campus, Khartoum North 13314, SUDAN

*Email for Correspondence: dr_nahla2004@yahoo.com

ABSTRACT

Mosquitoes are a large diverse group of insects, with members being most important as vectors of diseases. The correct identification of mosquitoes is crucial to the control of the mosquito-borne diseases. This study was designed to provide baseline data on the species composition and distribution of members of the Anophelines and Culicines genera in ten selected localities in Kosti region, White Nile State, Central Sudan. Global Positioning System (GPS) was used to select collection sites. Adult mosquitoes were collected by pyrethrum spray catch (PSC) from randomly selected houses in Allia, Alnsr, Alskahdeed, Almrabie, Alshati, Althwrat, Alraba, Block 26, Block 32 and Khadugli in Kosti region during September 2014-August 2015. The Anophelines and Culicines mosquitoes were identified using standard morphological keys. Two Anopheles species and three Culex species were morphologically identified. These were An. gambiae s.s.; An. pharoensis; Cx. quinquefasciatus; Cx. univittatus and Cx. pipiens. This is the first report of An. pharoensis in the study area. The highest mosquito abundance was observed in the September followed by October, while the lowest mosquito abundance was in July. An. gambiae s.s and Cx. quinquefasciatus were the predominant species which has importance as they indicate the presence of Malaria and arboviral infections in the study areas; respectively. These findings are of importance in the planning and implementation of vector control strategy in the Kosti region, White Nile state.

Key words: Mosquitoes, Morphology, Identification, Anophelines, Culicines, Kosti, Central Sudan

INTRODUCTION

Mosquitoes are among the most important disease vectors, known to transmit and maintain the circulation of pathogens that cause both global and neglected tropical diseases in humans and animals. The mosquitoes were originally all contained in four genera; Culex, Anopheles, Aedes and Corethra (Theobald, 1906). They have a significant role as vectors of many serious human and animal diseases such as malaria, yellow fever, encephalitis, lymphatic filariasis and Rift Valley fever (Anosike et al., 2007).

Of the 465 formally named anopheline species worldwide, about 41 (9%) are incriminated as malaria vectors (Harbach, 2013). However, Africa experiences the bulk of the global malaria burden due in part to the presence of the An. gambiae complex, which includes Anopheles gambiae, An. arabiensis, An. merus and An. melas. There are a further three, highly anthropophilic in Africa, An. funestus, An. moucheti and An. nili (Sinka et al., 2010). Nevertheless, An. arabiensis is considered the dominant and most dangerous malaria vector in Sudan. It is being reported from all parts of Sudan, and distributed over the dry savanna belt and semiarid parts of the country (Petrarca et al., 2000).

Mosquitoes of the Culex pipiens complex are cosmopolitan and important vectors of neglected tropical diseases, such as arbovirosis and lymphatic filariasis. Among the complex taxa, Cx. pipiens (with two forms pipiens and molestus)
and Cx. *quinquefasciatus* are the most ubiquitous mosquitoes in temperate and tropical regions respectively. Mosquitoes of this taxa lack of morphological differences between females, but have frank behavioral and physiological differences and have different trophic preferences that influence their vectorial status (Shaikievich et al., 2016).

Three genera of medically important mosquitoes are found in Sudan (*Anopheles, Culex* and *Aedes*). A number of 156 species, 2 subspecies of mosquitoes and 7 varieties of Culicidae have been recorded in Sudan. Forty five species of *Culex* were recorded in Sudan, the most prominent was *Culex quinquefasciatus* followed by *Cx. univittatus*. Another species of medical importance was mentioned some years ago, this was *Cx. poicilipes*. *Cx. quinquefasciatus* is the predominant species of *Culex* in Sudan. It is known to be a domestic annoying mosquito. It breeds abundantly in any collection of water such as barrels, tanks, culverts, etc. It can breed in fresh, brackish, and foul water such as cesspits and sinks. It is a vector of filariasis in Sudan and encephalitis abroad (El Rayah, 2007); also 38 species of Anopheles were recorded, and few species transmit malaria (Nugud et al., 1997). Mosquitoes of the genus *Culex* transmit West Nile Virus (WNV) and Rift Valley Fever (RVF). Lymphatic filariasis is caused by parasitic nematodes: *Wuchereria bancrofti*. In Sudan the disease is restricted to three regions in Kordofan, Darfur and the Blue Nile States, but now it tends to include most of the States. It is transmitted by *Culex* mosquitoes. More promising; the future potential distribution of *An. arabiensis* in Africa will be reduced as a result of climate change as revealed by the ecological niche modelling study (Drake and Beier, 2014). Although still below target levels, current malaria interventions (Insecticide treated bednet coverage (ITNs), Indoor residual spraying coverage (IRS), and Artemisinin-based combination therapy coverage (ACTs)) have substantially reduced malaria disease incidence across the continent (Malaria Atlas Project, 2017).

In order to correctly assign spatio-temporal distributions, behaviors and responses to interventions to particular anopheline species, identification of mosquitoes must be accurately made (Stevenson and Norris, 2016). The correct identification and description of mosquitoes’ vectors are crucial to understand the transmission of mosquito-borne diseases and to design the appropriate control strategy. Therefore, the main objective of this study was the identification of the mosquitoes’ composition and the relative abundance of each species in the study areas.

**Materials and Methods**

**Study area**

The study was carried out in Kosti, White Nile state in Central Sudan. It is the largest city in the state at an altitude of 390 meters above the West Bank of the White Nile and is located 360 kilometers south of the capital, Khartoum. Ten different locations, Allia (13.14745°N - 32.67914°E), Alnsr (13.15724°N - 32.67572°E), Alskahdeed (13.15839°N - 32.67871°E), Almrabie (13.16475°N - 32.67227°E), Alshati (13.17278°N - 32.66313°E), Althwrat (13.16200°N - 32.64886°E), Alraba (13.14029°N - 32.65282°E), Block 26 (13.15892°N - 32.66083°E), Block 32 (13.15138°N - 32.64664°E) and Khadugli (13.15313°N - 32.63335°E) were sampled for mosquitoes fauna. The topographical features of Kosti consisted of flat land interspersed with the northwest sand dunes locally known as “gayzan”. Soil is characterized as few mud spills and severe cracking in the drying period. The study region has generally hot dry climate with one long rainy season, which usually extends from June to September with small year-to-year variation and with a maximum temperature ranges between 41° C in April / May and 33° C in August and a minimum temperature ranges between 23° C in May and 17° C in January. In the period between October and May the wind blowing toward the region northeasterly and it rains an average of up to 350 mm per year. In the dry period the relative humidity rate is approximately 26%, while it is 71% in the rainy season. The main vegetation in Kosti is composed of *Acacia mellifera* (Kitir), *Acacia seyal* (Talih), *Acacia tortilis* (Sayal), *Salvador apersica* (Arak), *Commiphora africana* (Gafal) and *Maerua crassifolia* (Sareh) (Elsafori et al., 2012). People work as farmers, herdsmen, merchants and laborers. Domestic animals include ducks, poultry, cattle, sheep, goats, donkeys and horses with slight variation in the different collection sites. These sampling sites were selected and mapped using Global Positioning System (GPS). Houses are constructed from one / two of these materials: clay, bricks and cement with concrete roof or zinc covered with straw.

**Mosquitoes Collection**

Permission was obtained from the household heads before the start of the study. Adult mosquitoes were collected from both natural and man-made habitats, using pyrethrum spray catches (PSC), hand-landing technique, active search collection once per month for the period of study (September 2014 - August 2015). A collected mosquito sample was preserved in Silica gel, for morphological identification. In the hand collection method, mosquitoes were collected early in the morning. A separate cup was used for each site, and the collector was supplied with an aspirator, a torch and paper cups covered with mosquito netting. The cups were clearly labeled in pencil with the locality and date of collection. Mosquitoes were kept in the paper cups with a piece of moistened cotton on top to protect them from excessive heat and dryness until brought to the laboratory. The Spray sheet collection method was used for indoor-resting mosquitoes. The knock-down method was carried out early in the morning, usually between 7-10 am, of the day following the human-landing collection. The inhabitants of each room were first requested to vacate the room and...
then the whole floor was covered with white cotton sheets. The door, the windows and all possible mosquito exits were tightly close. The room was filled with a mist of 0.25 % solution of Cypermethrin (a pyrethroid) in water, using a medium-sized hand atomizer (spray-pump). It was left closed for 15 minutes. The dead mosquitoes were then picked off the sheets and collected on Petri dishes that contained moistened cotton covered with filter papers. Subsequently, they were taken to the laboratory for morphological identification with keys.

**Mosquitoes Identification**

A total number of 100 of the field-collected mosquitoes were randomly selected from each location in order to be identified by external morphology following the keys of the morphological identification of mosquito species of the Afro-tropical region. The mosquitoes were examined under the dissecting microscope using adult morphological identification key for Anopheles (Gillies and De Meillon, 1968) and *Culex spp* (Harbach, 1985). The morphological characteristics useful to distinguish between Anopheline and Culicine species such as wings, legs, thorax, abdomen, head and mouthparts for *Anopheles gambiae* complex and *Culex spp.* were observed using a dissecting microscope and permanent slides were prepared and photocopied in order to keep a record. The following characteristics were observed:-

**Anopheles gambiae s. s.:** These groups of mosquitoes have their abdomen without laterally projecting tufts of scales, the scaling on the abdomen was scanting and confined to the 8th or rarely 7th tergum. Legs are speckled with tarsi 1-4 having conspicuous pale bands on the apices. The palps were smooth or shaggy with pale bands and the 3rd preapical dark area on vein 1 with a pale interruption, which sometimes fused with proceeding pale spots.

**Anopheles arabiensis:** These groups of mosquitoes have their abdomen without laterally projecting tufts of scales, their hind tarsi 4 and 5 were entirely pale and palps with 3 pale bands usually with some specklings while veins I with 2 have accessory pale spots with speckled legs.

**Anopheles pharoensis:** These groups of mosquitoes have their abdominal segments with laterally projecting tufts of scales on segments 2-7. Shaggy palps, wing with abundant pale areas, costa with at least 4 pale spots and hind tarsus 5 and about apical half of 4 are pale.

**Culex univittatus Theobald:** These groups of mosquitoes have their wings with pale scales at base of costa. The hind tibia is with anterior pale stripe and the mid femur with complete anterior pale stripe.

**Culex quinquefasciatus Say:** These groups of mosquitoes have their abdominal terga with basal pale bands and the fore coxa with some dark scales, usually mostly dark. The wing is entirely dark scaled and the subcosta intersects costa before level of furcating of R₂+₃.

**Culex pipiens Linnaeus:** These groups of mosquitoes have their abdominal terga with basal pale bands. The wing is entirely dark scaled and the subcosta intersects costa at or beyond level of furcating of R₂+₃.

**Data analysis**

Chi (X²) square test was used to analyze and compare the differences in the species of the *Anopheles* and *Culex* mosquitoes in the study sites.

**RESULTS**

**Climatic results**

The meteorological climatic conditions of the study area “Kosti” during 2015; such as the maximum and minimum temperature is illustrated in Figure 1; monthly total rain fall is illustrated in Figure 2 and the percentage of monthly relative humidity is illustrated in Figure 3.
Figure 2: Monthly Total Rain Fall in the Study area during 2015

Figure 3: The Percentage of Monthly Relative Humidity in the Study area during 2015

*The average annual relative humidity is 45.1% and average monthly relative humidity ranges from 26% in April to 71% in August

Figure 4: Map showing the Sampling sites in Kosti region used in this study
Morphological identification results

Selected wing, leg, abdomen, and head characteristics were observed and analyzed on 1000 adult mosquitoes from 10 populations (Figure 4) using a dissecting microscope. All of the recognized features for both Anopheline and Culicine mosquitoes are illustrated in Figures 5 -17. All the morphologic terms and abbreviations used in these figures follow those of Gillies and De Meillon (1968) for Anopheles spp. and (Harbach (1985) for Culex spp. In the field collected mosquitoes, Anopheles was identified as having spotted wing and long palps, while Culex as having transparent wings and short palps. The male was distinguished from the female by the presence of plumose antennae. An. pharoensis is illustrated in Figures 5 and 6. An. gambiae s. s. is illustrated in Figures 7 – 10. Cx. univittatus is illustrated in Figure 11. Cx. quinquefasciatus is illustrated in Figures 12 – 14. Cx. pipiens is illustrated in Figure 15.

Figure 5: Wing (costa) with 4 pale spots in An. pharoensis

Figure 6: Shaggy palp in An. pharoensis

Figure 7: Palps with 3 pale bands in An. gambiae s. s.
Figure 8: 3rd main dark area of 1st vein with a pale interruption sometimes fused with preceding pale area in *An. gambiae s. s.*

Figure 9: Tarsi 1-4 with conspicuous pale bands on at least the apices in *An. gambiae s. s.*

Figure 10: Wing with pale fringe spots opposite all veins up to 5b or 6 in *An. gambiae s. s.*

Figure 11: Wing with pale scales at base of costa in *Cx. univittatus*
Figure 12: Abdominal terga with basal pale bands in Cx. quinquefasciatus

Figure 13: Forecoxa with some dark scales, usually mostly dark in Cx. quinquefasciatus

Figure 14: Wing entirely dark-scaled. Subcosta intersects costa before level of furcating of R2+3 in Cx. quinquefasciatus

Figure 15: Subcosta intersects costa at or beyond level of furcating of R2+3 in Cx. pipiens

Mosquitoes species abundance and distribution in the study areas

Out of 1009 mosquitoes sample collected in this study, 80.67% are Culex species and 19.33% are Anopheles species. Out of the 195 Anopheles 187 (95.90%) are Anopheles gambiae complex that includes Anopheles gambiae s.s. and An. arabiensis. The variation in the sibling species composition of An. gambiae complex was significant (p < 0.05). Anopheles gambiae s.s. was predominant in almost all localities and the only exception was Kadugli (Figure 18).
Out of the 814 *Culex* 403 (49.51%) are *Cx. quinquefasciatus* and 398 (48.89%) are *Cx. univittatus* and only 13 (1.60%) are *Cx. pipiens*. The species composition in each locality is illustrated in Figure 18. While the species distribution in the study areas during the period from September 2015 to August 2016 is illustrated in Figure 19. Female distribution is illustrated in Figure 20 and the male distribution is illustrated in Figure 21.

**Figure 16:** Map showing the Mosquitoes species distribution in sampling sites using Global Positioning System (GPS) in Kosti District

**Figure 17:** Mosquitoes species distribution during the period September 2015 – August 2016 in Kosti District, Central Sudan

**Figure 18:** Female Mosquitoes species distribution during the period September 2015 – August 2016 in Kosti District, Central Sudan
Statistical Results

The program “An interactive calculation tool for chi-square tests of goodness of fit and independence” (Preacher, 2001) was used for statistical analysis among mosquitos’ populations with N 1009. The chi-square statistic for all mosquitos was 368.669. The p-value was 0.00; the result is significant at p < .05. The chi-square statistic for female’s mosquitos was 295.339. The p-value was 0.00; the result was significant at p < 0.05. The chi-square statistic for male’s mosquitos was 114.923. The p-value was 0.00; the result was significant at p < .05. This result indicates that there was high significant variation among the distribution of the various mosquitos’ species within the 10 different localities in Kosti district.

Discussion

Identification of Anopheles and Culicine in the selected study areas in Kosti district had a total of two Anopheles species and three Culex species which were based on their morphological characters. These were An. gambiae s.s.; An. pharoensis; Cx. quinquefasciatus; Cx. univittatus and Cx. pipiens.

The report of An. gambiae s.s in Kosti district, Central Sudan during this study is in agreement with the previous reports from Africa, which stated that the most important members of the complex involved in malaria transmission are An. gambiae s.s., An. gambiae s.l., An. arabiensis. An. quadriannulatus, An. melas, An. merus; An. bwamba and An. funestus (Coluzzi et al., 1979; White, 1985; Gillies, 1987; Gillies and Coetzee, 1987; Coetzee et al., 1993; Hunt et al., 1998; Coetzee et al., 2000; Pock et al., 2003; Coetzee, 2004; Moreno et al., 2004). However, some of them are of minor importance. These species vary in relative frequency and distribution from one geographical location to another; distribution is influenced by prevailing climatic conditions (Lindsay et al., 1998).

In Sudan, there are 29 anopheline mosquito species that have so far been recorded (Lewis, 1958) they are widely distributed throughout the country, but more often are localized in the humid areas. An. arabiensis and An. gambiae are the only species of the An. gambiae complex reported in Sudan (Zahar, 1985; Petrarca et al., 2000). The identification of An. gambiae complex in Kosti district, White Nile state during this study is a confirmation of a previous study that reported An. gambiae arabiensis as the most frequent species (80.7%) in White Nile state (Seufi and Galal, 2010). This result is in agreement with previous ones that An. gambiae s.s is the most widely distributed species in Sub Saharan Africa, due to its genetic heterogeneity which enables it to adapt to many ecological zones (Service, 2014).

The entomological results showed that An. gambiae complex is most predominant species in all localities except Kadugli one, the only other Anopheline species in the study areas was An. pharoensis Theobald. These findings are in agreement with those of Lewis (1958) and El Gadal et al. (1985) who’s found, An. pharoensis from Gezira area, but this species play no role in malaria transmission because of their largely zoophilic behaviour (Lewis, 1958; El Gadal et al., 1985). The An. pharoensis was reported from three localities; Block-26, Alshati and Alnsr. The finding of An. pharoensis (Theobald, 1901) in these localities could be explained by the influence of modern means of transport in the distribution of mosquitos species in the country, since these three localities lie in close proximate to the railway line (Kosti – El Obeid). We suggest that this species is imported from other places to Kosti region.

The record of An. pharoensis in Kosti district, White Nile state was the first report of occurrence of this species of Anopheles in this area and this species was only reported previously with low density (0.1%) in Eastern Sudan (Himeidan et al., 2004) and in Gezira in Central Sudan (el Safi and Haridi, 1986). The low numbers of An. pharoensis in

Figure 19: Male Mosquitoes species distribution during the period September 2015 – August 2016 in Kosti District, Central Sudan
the indoor – resting collections may be attributed to the zoophilic nature of this species since there were considerable numbers of domestic animals that includes cattle, sheep, goat, donkeys, horses and poultry in the study areas.

The confirmation of An. gambiae s. s. as the predominant Anopheles species is important because it reveals the presence of the Malaria vector in the study areas. Another observation was that, An. gambiae s. s in all collections which means that an expected Malaria transmission may occur all over the year in the study areas.

The reporting of both Cx. quinquefasciatus and Cx. univittatus as the main Culex species in Kosti district was in consistence with the previous statement (El Rayah, 2007), that there were 45 species of Culex in Sudan, the most prominent was Cx. quinquefasciatus followed by Cx. univittatus. However, Cx. quinquefasciatus was absent only in the July collection,. It is known to be a domestic annoying mosquito. It breeds abundantly in any collection of water such as barrels, tanks, culverts, etc. It can breed in fresh, brackish, and foul water such as cesspits and sinks. This species is widely distributed in Asia, Africa and America. It is a vector of filariasis in Sudan and encephalitis abroad (El Rayah, 2007). It has been shown recently (Samy et al., 2016) that the highest stability of Cx. quinquefasciatus with climate change is observed between 30°S and 30°N and the finding of this mosquitoes species in Kosti district which lies in 13°10′N 32°40′E during this study confirms this statement.

The entomological results showed that Cx. quinquefasciatus followed by Cx. univittatus is most predominant species in all sites, the only other Culex species in the study area Cx. pipiens was the species found just in Almrabie. The presence of Culex in relatively high proportion reflects its wide feeding range. The observation that Cx. quinquefasciatus is the predominant Culex species is important as it reveals the presence of the Arboviruses vector in the study areas.

Overall, the September collection was the richest one in terms of the abundance of the 5 identified mosquitos’ species from Kosti district with the highest density of Cx. quinquefasciatus. While, July collection was the poorest one. The climatic conditions clearly influence the species distribution in the area. Variation in the annual distribution of the both sexes was observed. The houses construction, human activities, presence of broken pipes, water sources and type of soil, all had direct effect on the amount of the collections.

CONCLUSION

The study has revealed that, 2 different species of Anopheles mosquitoes found in the study area with An. gambiae s.s showing the highest prevalence over other species while An. pharoensis s was the lowest in population. In addition 3 different species of Culex mosquitoes were identified with Cx. quinquefasciatus and Cx. univittatus showing the highest predominance while Cx. pipiens was the lowest in population. The observation of An. gambiae s.s in Kosti district is of importance in malaria epidemiology. These findings are of importance in the planning and implementation of malaria vector control strategy in the Kosti region, White Nile state. We concluded that the morphological technique is suitable for preliminary field survey, particularly in a country of low setting resources.

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