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## Mapping of *Anopheles* mosquitoes (Diptera: Culicidae) in Gedarif State, Eastern Sudan

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### Abstract

*Anopheline* mosquitoes (Diptera: Culicidae) consist of a large number of species. The distribution (mapping) of malaria vector is important for strategic planning and disease control strategies. This cross-sectional study was conducted in Gedarif state (14-16 N and 33-36 E longitude; 75,000 km<sup>2</sup>, 1.8 million people), eastern Sudan, to identify *Anopheline* species and their breeding sites and habitats as part of a national mapping project. Mosquitoes were collected in the dry- and rainy- season, starting June 2014 to May 2015. Larvae were collected from the breeding sites by dipping and netting techniques. Adults were collected by aspirators, pyrethroids spray catches and Center for Disease Control (CDC) light traps from 9 stations. Morphological identification of adult specimens showed the presence of *A. arabiensis* (98.5%), *A. rufipes* (1.0%) and *A. pharoensis* (0.5%). *A. arabiensis* is the only member of *A. gambiae* complex predominant in all localities, co-existing with the other two species. Mapping of these vectors in all states must be a yearly task to assist in planning and decision-making.

**Keywords:** *Anopheles*, Gedarif, mapping, mosquitoes, Sudan

### 1. Introduction

Malaria spreads from one person to another by female mosquitoes of the genus *Anopheles* (Diptera: Culicidae) [1]. There are about 400 different species of *Anopheles* mosquitoes, but only 30 of these are vectors of major importance [1]. WHO estimates that there are approx. 3.2 billion people at risk of contracting malaria. Although the greatest proportion of the total at-risk population lives in Asia, malaria has the greatest health impact in Africa, which accounts for 80% of worldwide cases and 89% of deaths [2]. Malaria is one of the public health problems in Gedarif state, eastern Sudan. It exists in hypo- meso endemic zone of malaria (the parasite prevalence 10.2 according to malaria prevalence and coverage indicator survey, 2012 [3]. The main groups of malaria vectors in Africa are *A. gambiae*, *A. funestus*, *A. nili* and *A. vmoucheti*. Each of these comprise a complex or group of genetically distinct species that are similar in morphology, but vary in traits that affect their role in transmission of malaria [4, 5]. The most important of these groups is the *A. gambiae* complex, which comprises eight closely-related species that are distributed through sub-Saharan Africa (SSA), and its outer islands [6, 7]. In SSA, where 90% of the world's malaria cases occur, *A. gambiae* Giles, *A. arabiensis* Patton of the *A. gambiae* complex and *A. funestus* Giles from the *A. funestus* group are the most efficient malaria vectors [8, 9, 10].

*A. arabiensis* is the primary malaria vector throughout much of Sudan [11]. Other *Anopheles* species, such as *A. funestus*, *A. nili*, *A. pharoensis*, *A. rufipes* and *A. dhali* are also present in the country, but they play a negligible role in malaria transmission. Malaria transmitted by *A. arabiensis*, continues to be a major health problem in the Sudan [12, 13].

Mosquitoes in the eastern region of the Sudan were including *A. gambiae*, *A. funestus*, *A. rupicolus*, *A. pretoriensis*, *A. dhali*, *Culex nebulosus*, *C. sitiens*, *C. duttoni*, *C. univittatus*, *C. siniticus*, *C. laticinctus*, *C. fatigans (quinquefasciatus)*, *C. poecilipes*, *C. simpsoni*, *C. pipiens*, *Aedes aegypti*, *Ae. caspius*, *Ae. metallicus*, *Ae. vittatus*, *Ae. arabiensis* and *Ae. fowleri* [14].

Studying the distribution of malaria vectors is important for the strategic planning and disease control measures, in addition to mapping of the important species under the current climatic changes that might cause new distribution and introduction of new or invasive species to the same areas. Hence, maps can reveal spatial or geographical patterns of information. On the other hand maps are used in malaria control to support planning, management and decision-making was why present study was conducted.

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## 2. Materials and methods

### 2.1 Study Area

This study was conducted in Gedarif state (75,000km<sup>2</sup>), eastern Sudan, which lies entirely between latitude 14-16 N and 33-36 E longitude. Gedarif state plays a significant role in the economy and agricultural products of the country. The state is bordered by Khartoum state in north, Sennar state in the south, Kassala state and Ethiopia in the east and Gezira state in the western border. The state is divided in to 12 administrative localities (map). The population of the state is estimated as 1,827,181 consisting of most of the tribes of the country. The majority (65.9%) of the population works in agriculture. The vegetation of the state is poor savannah. The soil is clay and muddy. There are several rivers that pass through the state (*viz.* Atbara, Elrahad, Sitait and Basalam). Average of the rainfall is *approximately* 612 mm. More than 75% of houses in Gedarif state are huts. Several mountains and forests are scattered throughout the state. The forests are mainly composed of *Acacia* trees. Because of the summer heat, most of the inhabitants of the state sleep during the night in their houses yards, and sleep indoors, during the winter months or the autumn (Kharief), when it is raining. Safe water is available in towns and big villages with some shortage from March to June. But in rural and agricultural areas, people depend on large water reservoirs (Haffirs). A good communication system highways connect the state with the rest of the states and Port –Sudan in the Red Sea.

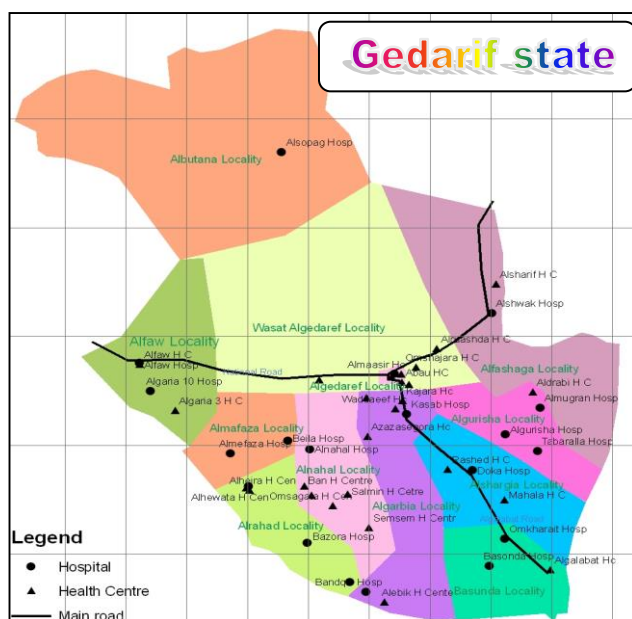


Fig 1

### 2.2 Sampling Strategy and Sample Size

Mosquito larval instars were collected from different breeding habitats (from five stations). Adults were collected from houses using the spray sheet method [15], and from the nine stations. Sampling was made within a radius of approx. 1 km from the center of the station. Three dips, using the scooping net methods were taken arbitrarily at random from each positive site for larval collection, and five rooms were selected [16] randomly in each station for adult collection. The collection is carried out between 8:00 am to 10:00 a.m. [17]. Center for Disease Control (CDC) light trap was used to collect mosquito populations outdoor from the same houses

selected for indoor collection. The number of captured mosquitoes was recorded. Larvae were transported to the laboratory, washed and killed with hot water (50°C–70°C)18, kept in 70% alcohol and mounted on slides using Euparal [17].

### 2.3 Morphological Identification

The collected larvae and adults were identified by morphological characters using the *Anopheles* mosquito keys of the Sudan for larvae and adults [8, 9, 19]. The 4<sup>th</sup> instar larvae were mounted [20] and classified using the keys of Hopkins [21], Ralf [22] and Nugud [19], in addition to the software key of the *Anopheline* mosquitoes of the Afro-tropical Region. The main features for classification were abdomen, tarsomere, palpi and legs.

### 2.4 Data Analysis

The data were analyzed using SPSS software Program, ANOVA (Ver.16). The descriptive analysis was used, the data was analyzed step by step, and tabulated to explain the possible relationship between the variables.

## 3. Results

### 3.1 Adults Collection and Identification

A total number of 1553 adult specimens representing three *Anopheline* species were recorded for both the dry- and rainy-seasons collected by the aspirator and Pyrethrum Spray Sheets. The total percentage of adult species identified morphologically, as mentioned above, were: *A. arabiensis* (98.5%), *A. rufipes* (1.0%) and *A. pharoensis* (0.5%). The highest number of adult was collected from Doka sentinel site (426), while Galabat sentinel site registered the lowest number (30 adult). Tabarakalla, Galabat, Alshowak, Hilat Khatir and Gedarif collected adults proved to be as *A. arabiensis* only (100%), while the other three sentinel sites (*viz.* Doka, Alfau and Alhowata), in addition to this species, also proved to include the other two species, *i.e.* *A. rufipes*, and *A. pharoensis* (Table 1). The results showed density of *Anopheline* mosquitoes in rainy -season was significantly higher (1,352) than the dry-season.

### 3.2 Larval Stage Collection and Identification

With regard to the larval stage, a total of 249 *Anopheline* larvae were collected from 5 locations during the period of June 2014 to May 2015 from Gedarif sentinel site. The larvae were categorized into late-instars (L3 and L4). *A. arabiensis* was the only species in these areas (Table 1). The highest density of the larvae /10 dips was 4.8 in Doka sentinel site, followed by Albutana sentinel site (3.7), Alshowak (2.7), Gedarif (1.9) and Tabarakalla showed the lowest density (1.2 larvae/ 10 dips; Table 2).

### 3.3 Pyrethrum Spray Sheets Collection Method

The highest number of adults caught using pyrethrum spray sheet was found to be 13.8 adults /room in Alfau sentinel site. While Galabat sentinel site registered the lowest number of adults (1.0/room) during the same month. Moreover, October registered the highest density of adult mosquitoes in all sites, while April and May proved to be free from adults. During February, all sites were free from adults with the exception of Gedarif and Alfau with a density of 0.2 adults/room for each (Table 3)

**Table 1:** Total number of adults of *Anopheles* spp. caught by Pyrethrum Spray Sheets in Gedarif State, June2014-May2015.

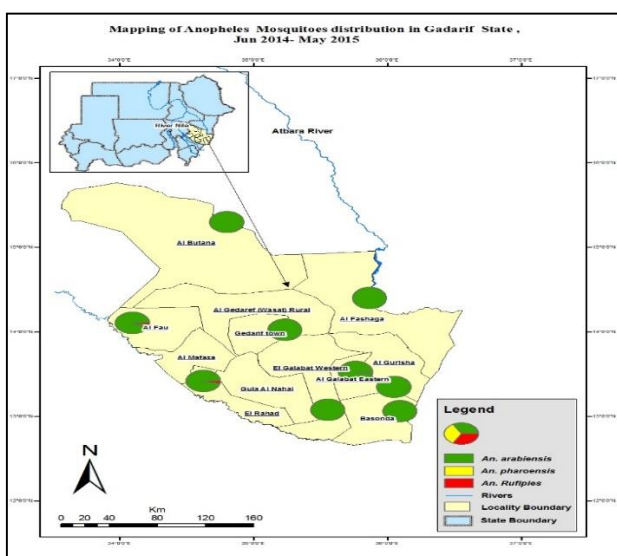
Anopheles species Sentinel site	<i>A. arabiensis</i> No (%)	<i>A. rufipes</i> No (%)	<i>A. pharoensis</i> No (%)	Total
Doka	420 (98.60)	1 (0.23)	5 (1.20)	426
Tabarakalla	104 (100)	-	-	104
Galabat	30 (100)	-	-	30
Alshowak	319 (100)	-	-	319
Alhowata	105 (96.3)	4.0 (3.7)	-	109
Hilat Khatir	41 (100)	-	-	41
Gedarif	329 (100)	-	-	329
Alfau	182 (93.3)	8 (4.10)	5.0 (2.6)	195

**Table 2:** Distribution of *Anopheles* larvae collected in Gedarif sentinel sites and their percentages for the period from June 2014 to May2015 (by dipping method).

Sentinel site	No. of <i>A. arabiensis</i>	%
Doka	91	36.54
Tabarakalla	12	4.81
Galabat	0	0.00
Alshowak	49	19.67
Alhowata	0	0.00
Hilat Khatir	0	0.00
Gedarif	24	9.63
Alfau	0	0.00
Albutana	73	29.31
Total	249	

**Table 3:** Adult *Anopheles* species density /room and /months in Gedarif State for the period from June 2014- May 2015 (Pyrethrum-spray sheet method)

Month	Doka	Tabarak-alla	Galabat	Alshowak	Alhowata	Hilat Khatir	Gedarif	Alfau	Total
Jun	6.0	0.0	0.0	0.2	0.4	0.0	0.6	1.4	8.6
Jul	4.2	2.4	0.0	1.2	2.6	0.0	2.0	1.4	13.8
Aug	3.8	4.8	0.0	1.6	3.2	0.8	5.4	3.2	22.8
Sep	6.6	5.4	2.6	4.2	3.8	2.6	4.7	3.0	3.9
Oct	9.2	6.2	2.4	4.8	4.2	3.0	7.4	11.2	48.4
Nov	8.0	1.4	1.0	6.0	6.2	1.8	4.4	13.8	42.6
Dec	4.0	6.0	0.0	1.2	0.6	0.0	5.0	4.0	20.8
Jan	2.0	0.0	0.0	0.0	0.2	0.0	0.0	0.8	3.0
Feb	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.4
Mar	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.6
Apr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0



**Fig 2:** Map by GIS for Gedarif State showing the sentinel sites and *Anopheles* species distribution in the state.

**4. Discussion**

Eleven species of *Anopheles* were found in the Sudan, viz. *A.*

*arabiensis*, *A. gambiae*, *A. funestus*, *A. nili*, *A. dthali*, *A. squamosus*, *A. rufipes*, *A. pharoensis*, *A. pretoriensis*, *A. coustani*, and *A. multicolor* [23]. Elrayah [14] in the eastern region of the Sudan, including Gedarif state, identified *A. gambiae*, *A. funestus*, *A. rupicolus*, *A. pretoriensis*, *A. dthali*, nine *Culex* species, and six *Aedes* species. In the present work, a total of 1,553 adult specimens were captured and found, using the morphological keys, to represent three species *Anopheles* during the dry- and the rainy- seasons of 2014, viz. *A. arabiensis*, *A. rufipes* and *A. pharoensis*. *A. arabiensis* is the most wide-spread member of the *A. gambiae* complex in the Sudan [25]. This species as *A. gambiae s.i* was reported as a major vector in the capital of the Sudan (Khartoum State) by several investigators. The species was found in a wide variety of places and habitats, principally in small pools, at least partially exposed to direct sunlight [24]. This vector is highly endophilic, except in Khashm El Girba area in Eastern Sudan, where the population was found exophilic. *A. arabiensis* was found to have a high vectorial capacity and, consequently, an efficient malaria vector [23]. As mentioned earlier, Abuelmaali *et al.* [11] stated that this species is the primary malaria vector throughout much of Sudan. Malaria is a major health problem in the

Sudan [12, 13]. Other *Anopheles* species, e.g. *A. funestus*, *A. nili*, *A. pharoensis*, *A. rufipes* and *A. dthali* are also present in the country, but they play a negligible role in malaria transmission [11, 12, 13, 14, 14, 23, 24, 25].

The results showed that the highest percentage of adults in the Gedarif state belong to *A. arabiensis* (98.5%). It is dominant in all the administrative localities of the State. However, *A. rufipes* population did not exceed 1.0%, and *A. pharoensis* was around 0.5% of the total mosquitoes population. The latter species is widely distributed in the eastern neighbor Ethiopia and its eastern neighbor Somalia; the species is also extends into Egypt in the north [26].

The invasion of the new detected species (*A. rufipes* and *A. pharoensis*) to the Gedarif State might be attributed to the huge modifications caused by the anthropogenic activities or climate change, in addition to the increased human population, their movement and activities. This may contribute in the spread of these species through the passive dispersion from the neighboring Sudanese state Gezira and Sennar.

The highest density of *Anopheles* species was recorded in Alfau sentinel site (13.8). This is probably due to the broken water pipes, in addition to its location within the irrigated Elrahad Agricultural Corporation, where canals are almost full of water throughout the year.

Population density of this species varied seasonally in relationship to rainfall. The density increased quickly with the first rains. and the maximum density was reached at the end of the rainy-season [27, 28] and, then decreased as the temporary breeding sites dry up. This species is known to be anthropophilic, however, at a lower frequency than *A. gambiae* [9]. The species is also known to have both endophilic (indoor resting) and *exophilic* behavior (resting outdoor), e.g. eastern Sudan [29]. Rainy-season in Gedarif state is from July in to October. The results indicated that the *Anopheles* captured in the rainy-season period in Gedarif state (1,352 adults), were significantly higher than those captured during the dry-season (201 adults). Moreover, the entomological surveys showed April and May to be free from the adults. Adults reached the highest density in October, which can be attributed to the cessation of the rainfall. The latter results in a continuous removal or washing of the breeding sites. The latter's formation starts from mid-September to November. Galabat and Hilat Khatir localities, in first rains, proved to be free from adults. This can be attributed to the rocky nature of the area, and the presence of many seasonal watercourse (Khors) or natural drainage of water. This is in an agreement with several authors [9, 27, 28, 29].

The availability of suitable breeding habitats depends not only on rainfall, but also on soil type moisture, texture, anthropogenic activities, human and animal populations, and human activities (agriculture, construction, etc.). Man-made ecological changes can lead to the formation of new ecological settings. Subsequently, there are changes in malaria vector species abundance, distribution and the pattern of the transmission [30, 31].

During February (winter), all site were free from adults, with the exception of Gedarif and Alfau with a density of 0.2 adults/room for each. This suggested that the heavy population and the human activities, in addition to some practices by the towns inhabitants vs. villages inhabitants could be encouraging the continuous presence of mosquitoes, e.g. broken network of water pipes, evaporative coolers, water storage containers, etc.

According to WHO [32], seasonality in malaria is usually due to fluctuations in environmental conditions (temperature, availability of breeding places and humidity). In tropical countries, the temperature is in favor of mosquito vectors throughout the year. However, in these areas, the transmission depends on the availability of the breeding sites, which in turns depends on the annual rainfall patterns.

*Anopheles* immature stages were available in the five localities of the Gedarif State. This could be attributed to the availability of water during the rainy-season, availability of the habitats, e.g. cistern leakage and broken water pipes exposed to sunlight and preferred by *A. arabiensis*. The results of the present study are consistent with Gillies and Coetzee 9. Gedarif climate allows the presence and the breeding of all *A. gambiae* complex, because of the optimum habitat for the mosquitoes. There may be a new species such as *gambiae s.s* and *A. funestus*, especially in the East Galabat and Elrahad regions.

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