

Identification and Classification of Sand Flies Species and It's Habitats in El-Kadaba Village, White Nile State, Sudan

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Abstract: Sand flies are a group of dipterans insects belonging to the subfamily Phlebotomine, family Psychodidae. Members of sand flies are delicate and can be easily recognized by their brownish colour, small size (1.5-2.5 mm), hairy appearance, long slender legs, jerky flight pattern and the characteristic manner in which they hold their pointed wings at an angle of 45° above their body. In this study, an entomological survey was carried out during June 2008 in El-Kadaba village (White Nile State, Sudan) to determine sandfly fauna and to identify sand flies vector(s). Sandflies were collected using sticky paper and light traps. Ten species of sandflies were recorded, three *Phlebotomus* species and seven *Sergentomyia* species of these sandflies, *P. rodhaini* was collected only from *Acacia seyal/Balanitiesaegyptiaca* at the Island whereas *S. huntii* was collected from the *Acacia nilotica* forest only of the total collections, *P. orientalis* representing 3.80% (248 specimens) of the total collection and *P. papatasi* were 3.11% of the collection. The abundant species recorded in the area were *S. squamipalpus* (39.76%), *S. clydei* (25.89%) and *S. antennatus* (12.39%). *Phlebotomus orientalis* were collected significantly in higher numbers from the Island (*Acacia/Balanities* thicket) (5.50 ± 0.98), than the village (3.25 ± 0.72). According to results of this study we recommended the following: more studies must be done in future to cover all the belt of sand flies to determine all species of sand flies in Sudan. Annually Entomological surveys must be done to determine density of Sandfly Vectors and Encourage the use of personal protection tools (ITNs), repellents, and improving of houses to avoid bite of sand fly.

Keywords: Habitats, Trap, Significant, Collection, Leishmaniasis, El-Kadaba Village

1. Introduction

Sandflies are grouped in six genera namely *Phlebotomus*, *Sergentomyia* and *Chinius* in the Old world and *Lutzomyia*, *Brumptomyia* and *Warileya* in the New World [1]. About 800 or so species of sandflies exist, only about 70 species of *Phlebotomus* and *Lutzomyia* are anthropophagous and thought to be involved in transmission of disease to man [2]; [3].

Sandflies are known to have a low flight pattern and they are usually collected from low height above the ground level. For example it has been found that *Phlebotomus argentipes* do not rest more than 1.5 meters above the ground level [4].

Little is known about the life history of different sandfly species in their natural habitat and the available information on immature stages of sandflies has been gained only from laboratory colonies of sandflies. Breeding sites of sandflies often takes place in damp places such as soil cracks, wall and rock crevices, animal burrows and caves and damp leaf litter in forests [5]. The main requirements for sandfly breeding sites are optimum temperature, moisture and presence of organic detritus on which larvae can feed.

Sandflies are typically crepuscular or nocturnal, biting at

different times of the night according to the species but, they will bite during the day when distributed (e.g. in dense forest, caves or buildings). Only a few species are endophilic; these are mostly peridomestic species, such as *P. papatasi*, *P. chinensis*, *P. sergenti* and *Lu. longipalpis*. During the daytime, they rest in dark humid places. The domestic species are frequently found resting in human dwellings between cloths, behind cupboards and hanging pictures as well as in the wall crevices. The wild species are often found resting in damp microhabitats such as rock crevices, caves, cracks and fissures in soil, bank of streams, animal burrows and leaf litter in forests. The main requirements for sandfly resting sites are optimal physical conditions such as still air, optimum conditions of temperature (28°C) and high relative humidity [5].

Both males and females feed on sugars for general activities i.e. flight, mating, and also sugar is essential for the full development of *Leishmania*. Sandflies obtain sugar meal from aphid honeydew or directly from plants by active piercing of leaves and stems [6]. In addition, female sandflies need vertebrate blood to mature and lay eggs during their gonotrophic cycle. Most species are gonotrophically concordant, taking one blood meal for each batch of eggs matured, although, some species are autogenous i.e. they can develop and mature eggs without taking blood-meals, e.g. *P. papatasi* [7]. Between 30-70 eggs are laid by a single female and hatching occurs one to two weeks later, depending on the environmental conditions [2]. At 28°C, the larva hatches from the egg and start to feed on organic matter [8]. The highest parity rates occur in sand flies population that associated with the end of peak of transmission. Although it is not easy to determine accurate of parity rates, the follicular relics methods were used to distinguish parous and nulliparous sandflies.

There are four larval instars of sandflies which feed on organic detritus and micro-organisms. The larva develops in 30-40 days into a pupa which is emerge into a fully grown adult in one week, approximately [8]. The period from oviposition to adult eclosion is 25-60 days, but may extend up to several months in diapausing species [9]. The adult emerges from the pupa during the hours of darkness often just before dawn. In males, the terminalia rotate through 180° during the first 24 hours after emergence. The age of female sandflies is measured by the number of gonotrophic cycle they undergo. Most species are gonotrophically concordant, but *P. papatasi* may feed more than once in an ovarian cycle.

Sandflies occur in a very wide range of habitats from sea level (around the Dead Sea which is below Sea level) to altitude of 2800 m or more in the Andes and Ethiopia, and from hot dry deserts, through Savannas and open woodland to dense tropical rain forest. Therefore, they exist in the tropics and subtropics, with a few species penetrating in to temperate regions in both the northern (50°N) and southern hemispheres (to about 40°S) [2].

In general, each species has fairly specific ecological requirements and in a few cases these encompass the conditions in and around the dwellings of man or in his

domestic animals. Many authorities differentiate "wild" and "domestic" species of sandflies, the latter being those found frequently in human dwellings, while the former are found mainly in outdoor situations and rarely if ever enter houses. For example in central Asia sandflies are restricted to the natural foci provided by colonies of gerbils (murid rodents of the sub family Gerbillinae) and by soil texture and moisture [2]. However, sandflies ecology relatively varies from animal burrows, caves or rock piles to human dwellings and distinction between peridomestic and 'wild' species therefore has little meaning in sparsely populated areas.

The abundance of sandflies in different habitats is mainly influenced by climatic and environmental conditions; these are temperature, rainfall, soil type and vegetation. In most of the Old World, adult phlebotomine sandflies are found only during the summer months, with populations of certain species peaking in late spring [10], whereas others tend to peak later on in summer [11]. Moreover, a major factor with which the sandfly appears to be highly associated is soil types. The association, also noted by Ashford and Bettini (1987), may be due to an indirect influence of soil type on the local microclimate, vegetation and other fauna, for example the associations of sandflies with coffee plantation in South America [6] and *Acacia seyal* in Sudan [12].

Most of man-biting sandfly species prefer to bite and rest outside (exophilic) near their breeding and resting sites. Sandflies biting activity for instance does not usually take place below 20°C. However, in tropical species, *P. papatasi* is active between 4 and 60 RH% but other species do not below 75-85 RH%. Also, the abundance of man-biting sandflies is influenced probably by the distribution of preferred hosts [2].

In Sudan, nine *Phlebotomus* and 37 *Sergentomyia* species have been recorded [13]. These *Phlebotomus* sandflies are *P. rodhaini*, *P. orientalis*, *P. pedifer*, *P. alexandri*, *P. saevus*, *P. papatasi*, *P. bergeroti*, *P. duboscqi*, and *P. martini*. Out of these species only two are thought to play a major role in disease transmission, namely; *P. orientalis* the proved vector of VL [15]; [16]; [17]; [18] and the incriminated *P. papatasi* as a vector of CL [19].

In Sudan, *P. orientalis*, the only proven vector of VL is mainly associated with *Acacia-Balanites* forests in endemic areas of kala-azar in the Eastern Upper Nile Province in southern Sudan [11]. This finding was confirmed by [20] and [21]. Similarly, in eastern Sudan *P. orientalis* was collected from forest habitats and villages [22]; [23].

Phlebotomus papatasi, the incriminated vector of cutaneous leishmaniasis in Sudan was found to be prevalent in CL endemic areas [24]. Other *Phlebotomus* species such as *P. sergenti* the vector of *L. tropica* had been previously caught in eastern Sudan [25], whereas, *P. duboscqi* the vector of CL in West Africa was found in different places in Sudan [24]. Although its role in transmission of CL in Sudan is unknown. *Phlebotomus rodhaini* naturally infected with *L. donovani* DNP was reported by [15], although its role in transmission of VL has not been proven.

2. Material and Methods

Study Area

El-Kadaba village is located on western bank of the White Nile River (White Nile State) ($32^{\circ} 14' E$, $14^{\circ} 72' N$), it distances about 200 km south of Khartoum (capital of Sudan). The area is flat and covered by an alluvium of silt

clay soil of the two types, black cracking clay soil and sand deposited by the river. Climate of it resembles a semi desert region therefore it is part of the arid climate with three distinct seasons; winter (November-February), summer (March-June) and autumn (July-October) with an estimated annual rainfall of <400 mm.



Figure 1. Map showing location of the study area.

Sample Size

In this study about 6527 sandflies were collected from different habitats during all the period of survey.

Data Collection

Light traps

The light traps used to collect nocturnally active sandflies were CDC miniature light traps (Model 512, John Hock Co.,

Gainesville, Florida, USA), connected to 6volts rechargeable batteries and sandflies cages (Plate 2). The light traps were generally hanged at 30 cm above the ground level at outdoor and indoor sites, between 18:00-06:00 HR. Captured sandflies were separated from other insects and preserved in 70% alcohol for identification.



Figure 2. CDC miniature traps used for sandflies collection during June 2008.

Sticky paper traps

The sticky paper traps were xerox paper sheets ($15 \times 21\text{cm}^2$) coated on both sides with diesel oil or castor oil and fixed vertically on sticks held at 15 cm above the ground (Plate 3) and left overnight from 18:00-06:00 HR and collected by the next morning. Sandflies found stuck on the oiled papers were removed using small brushes, washed in dilute detergent and preserved in 70% alcohol for identification.



Figure 3. Sticky paper traps used for sandflies collection during June 2008.

Data Analysis Obtained Data was analyzed by a computer through the program (SPSS) and excel programme. Then presented in tables and figures.

Table 3. Comparison between the abundance of *Phlebotomus orientalis* in study area during June 2008.

Site of collection of sandflies	M±SE Number of <i>P. orientalis</i> collected per sticky paper trap				Total	Total
	Male		Female			
	NO	M±SE	NO	M±SE	NO	
Village	19	1.50±0.20	21	3.25±0.72	40	4.75±0.94
Island	44	3.50±0.51	58	5.50±0.97	102	9.00±0.1.46
Forest	23	2.67±0.451	21	2.22±0.61	44	4.89±0.96
Open-land	29	2.50±0.43	33	3.00±0.58	62	5.50±0.92
P value		0.064		0.01		0.021
Total	115		133		248	

N= 248

3. Results

Ten species of sandflies were recorded, three *Phlebotomus* species and seven *Sergentomyia* species namely: *Phlebotomus papatasi*, *P. orientalis*, *P. rodhaini*, *Sergentomyia clydei*, *S. schwetzi*, *S. antennatus*, *S. squamipleuris*, *S. africana*, *S. sintonious* and *S. hunti*.

Table 1. Species of sand flies collected from study area during June 2008.

Species	Numbers of sandflies collected from the area	%
<i>P. orientalis</i>	248	3.79
<i>P. rodhaini</i>	21	0.32
<i>P. Papatasi</i>	203	3.11
<i>S. squamipleuris</i>	2595	39.76
<i>S. clydei</i>	1690	25.89
<i>S. schwetzi</i>	809	12.39
<i>S. africana</i>	302	4.62
<i>S. antennatus</i>	494	7.87
<i>S. sintonious</i>	101	1.55
<i>S. hunti</i>	64	0.98
Total	6527	100

N= 6527

The above table shows that 39.76%, 25.89% and 3.79% species of sand flies are *S. squamipleuris*, *S. clydei* and *P. orientalis* respectively.

Table 2. Sand fly species collected from different habitats in El-Kadaba village and the surroundings (White Nile) during June 2008.

Species	Mean±SE / oil trap				NO
	Village	Island	Forest	Open-land	
<i>P. orientalis</i>	3.25±0.72	5.50±0.98	2.22±0.61	3.00±0.58	248
<i>P. papatasi</i>	0.75±0.34	2.17±0.66	2.67±0.21	4.75±1.17	203
<i>S. squamipleuris</i>	11.25±1.12	67.33±9.15	22.44±2.26	28.25±1.75	2595
<i>S. clydei</i>	7.75±1.83	37.00±7.50	10.78±1.06	19.25±2.75	1690
<i>S. schwetzi</i>	5.00±0.48	10.67±1.68	8.11±1.06	18.00±2.74	809
<i>S. Africana</i>	0.00±0.00	6.17±1.27	2.11±0.34	4.00±1.21	302
<i>S. antennatus</i>	0.00±0.00	15.67±3.36	4.44±1.10	6.00±1.10	494
<i>S. sintonious</i>	0.00±0.00	1.50±0.70	0.00±0.00	1.25±0.56	101
<i>S. hunti</i>	0.00±0.00	0.00±0.00	1.33±0.42	0.00±0.00	64
Total					6506

N= 6506

The above table shows that species of *Phlebotomus* are collected from all targeted habitats while *Sergentomyia* species are collected from some habitats (i.e) *S. hunti* collected just from forest.

The above table shows that species of *Phlebotomus* female are more than male in all targeted habitats except in forest

Table 4. Density of sandfly species in two habitats of the Island (woodland of *A. seyal/B. aegyptiaca* and grass Island near the river) in study area during June 2008.

Species	Species and Mean±SE / oil trap					P. value
	A. seyal/B. aegyptiaca		Grass of Island near the river		Total	
	NO	M±SE	NO	M±SE		
<i>P. orientalis</i>	5	0.40±0.18	41	4.00±1.21	46	0.00
<i>P. papatasi</i>	20	2.40±0.11	17	2.00±0.56	37	0.00
<i>S. squamipleuris</i>	330	28.80±3.25	165	16.25±2.16	495	0.00
<i>S. clydei</i>	125	9.60±1.13	255	13.50±1.97	380	0.07
<i>S. schwetzi</i>	40	6.80±0.66	137	8.00±2.43	177	0.60
<i>S. Africana</i>	9	0.40±0.18	77	3.25±0.62	86	0.00
<i>S. antennatus</i>	0	0.00±0.00	192	11.00±1.34	192	0.00
<i>S. hunti</i>	0	0.00±0.00	64	4.00±0.82	64	0.00
Total	529		948		1477	

N= 1477

The above table shows that species of *Phlebotomus* are indicated statistical significant according to P. value while some of *Sergentomyia* species are indicated statistical significant.

4. Discussion

The results obtained on the sandfly composition in the study area generally agree with that reported from different areas in eastern, southern and northern Sudan (Quate, 1994; El-Sayed et al., 1991; Elnaïem et al., 1997). However, the collection of *P. orientalis* and *P. rodhaini* in this study add to the findings of Hassan et al. (2007) who reported the presence of the two species in northern Sudan for the first time. Although *P. orientalis* density fluctuated depending on habitat and the vegetation cover, the species were collected from several villages during the previous surveys. The finding is significant since *P. orientalis* is the main vector responsible for transmission of *L. donovani* which cause VL in eastern and southern Sudan (Ashford et al., 1992; Elnaïem et al., 1997; Hassan et al., 2004).

The most important sandfly species collected from the area in this study were *P. papatasi*, *P. rodhaini* and *P. orientalis*. *Phlebotomus papatasi* is the only vector of cutaneous leishmaniasis for being the man-biting species found in the endemic areas in northern parts of Sudan, (El Sayed et al., 1991). Although *P. rodhaini* is not known as a vector of leishmaniasis, recently it was found infected with *L. donovani* in the woodlands of DNP (Elnaïem et al., 2001). It is thought to play a role in maintaining the zoonotic cycle between reservoir animals, but does not transmit the parasites to humans.

Phlebotomus orientalis is the only proven vector of *L. donovani* in Sudan (Ashford et al., 1992; Hassan et al., 2004). *Phlebotomus orientalis* has been reported to be commonly occurring in endemic areas of VL within the savannah regions that provide certain environmental determinants of temperature, relative humidity and rainfall

(Elnaïem et al., 1998a). This species was not collected from semi-arid areas of northern Sudan although some VL cases were reported from Khartoum area and White Nile area during 1970s and 1980s (Hamza et al., 1976; Ahmed et al., 1988).

The results on the prevalence of sandfly species in different habitats in the area including the village habitat, the forest of *A. nilotica*, open land and the woodland of *A. seyal/B. aegyptiaca* indicate that *P. orientalis* was more abundant inside the *A. seyal/B. aegyptiaca* than the other habitats which suggest that the transmission of VL might take place in this habitats. This finding supported the results obtained by Elnaïem et al. (1997) in eastern Sudan who suggested that the transmission of VL takes place in woodland of *A. seyal/B. aegyptiaca* due to the sylvatic nature of the vector. *Phlebotomus orientalis* was reported to actively transmit *L. donovani* in eastern and southern Sudan during the same period (Ashford et al., 1992; Hassan et al., 2004). The fact that the activation of the recent outbreak could be due to recent agricultural activities practiced by the villagers in the sandfly infested area in the Island dominated by *A. seyal/B. aegyptiaca*. The villagers cultivate an Island within the *A. seyal/B. aegyptiaca* in the dry season during March-June. They move to the area with their families and children unprotected from the biting of insects. However, *P. orientalis* was collected in few numbers from the village habitats which might support the idea of possible in-village transmission of the disease. In village in eastern Sudan *P. Orientalis* was found infected with *L. donovani*. This indicates an active transmission inside these villages (Elnaïem & Osman, 1998; Hassan et al., 2004) but with very low rates.

The results on the indoor and outdoor prevalence of sandflies in El-Kadaba village show that *P. orientalis* were collected in low numbers only from outdoor sites. This finding indicates that this species is exophilic. Similarly, *P. orientalis* was collected only from outdoors in villages in eastern Sudan (Quate, 1964; Elnaïem et al., 1997; Lambart et al et al., 2002).

The morphological features of *P. orientalis* collected during this study were similar to those of the corresponding species reported by Kirk and Lewis (1951) and Quate (1964). The record of *P. orientalis* vector in the northern Sudan was a unique *phlebotominae* finding in this area. The presence of this sandfly vector which typically inhabits savannah environments in northern Sudan is very interesting from a biogeographical distribution viewpoint. Therefore, in the future more systematic studies are needed to determine their geographic distributions, to compare their seasonality and to elucidate their transmission potential. Also, the presence of *P. orientalis* in northern Sudan could indicate continuous changes in the environmental determinants of this vector species and the possibility of new foci of VL in Sudan in the future.

5. Conclusion

The sandflies species found in the area consists of

three *Phlebotomus* and seven *Sergentomyia* species which are namely *P. orientalis*, *P. papatasi*, *P. rodhaini*, *Sergentomyia clydei*, *S. schwetzi*, *S. antennatus*, *S. squamipleuris*, *S. africana*, *S. sintanious* and *S. hunti*. *S. squamipleuris*, *S. clydei* and *S. schwetzi* were the most dominant species in the area.

P. orientalis were collected from all habitats in the area, and *P. rodhaini* was collected only from the woodland of *Acacia seyal*/ *Balanites aegyptiaca* in the Island near El-Kadaba village, while *P. orientalis* was absent from indoor sites of El-Kadaba village.

Recommendations

According to results of this study it is recommended that:

More studies must be done in future to cover all the belt of Sandflies, Annually Entomological surveys must be done to determine density of Sandfly Vectors by seasons, Encourage the use of personal protection tools (ITNs), repellents, and improving of houses to avoid bite of sand flies and more studies are needed in the future to determine the transmission season, and infection rates of *Leishmania* parasites in human and the animal host in this area.

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