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A morphological study of phlebotomine sandflies and their role in the transmission of human leishmaniasis, with special reference to Namibia

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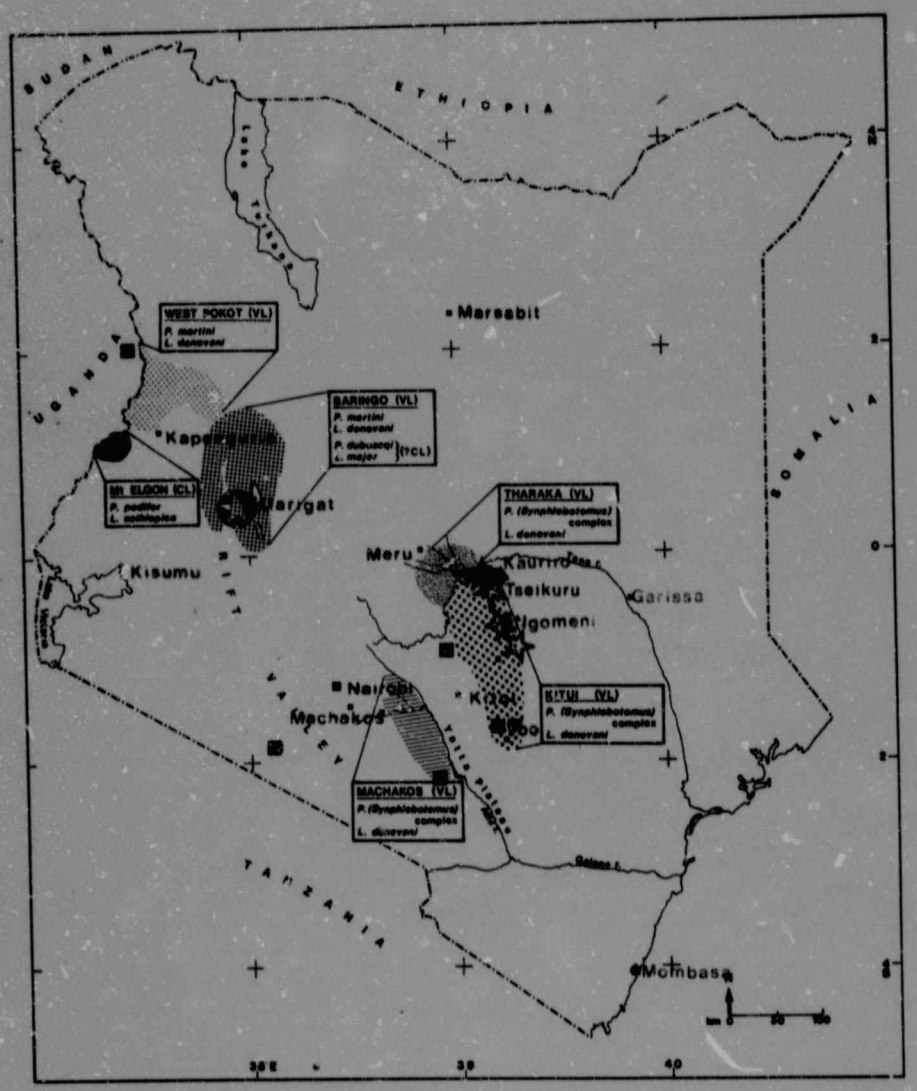
Fig. 3. Map of Kenya showing the distribution of VL and CL foci. Overlays show the distribution of the proven and potential **Phlebotomus** vector species.



LEGEND

▲ P. celiae ★ Type locality

▼ P. vansomerenae ☆ Type localities

■ P. martini ✪ origin of USAMRU colony

Fig.3

been recorded from SE Kenya. The females of the 3 species are indistinguishable nter, 1962; Killick-Kendrick & Ward, 1981; Kaddu, 1986) but the males are easily separable on terminalia differences. This similarity of the females causes identification problems especially in SE Kenya where all three species occur sympatrically. Entomological investigations in the West Pokot and Baringo districts (Mutinga **et al.**, 1984; Mutinga & Ngcka, 1978; Mutinga & Kamau, 1986), indicate that the only species belonging to the East African **Synphlebotomus** complex that has been collected is **P. martini**. In the Kitui district all three species of this complex are known to occur (Minter, 1962; Heisch **et al.**, 1962) and one can possibly assume that these species may also be prevalent in the adjacent Meru (Tharaka location) and the Machakos districts including the Masinga focus (Machakos district) which lies between the Kitui and Machakos foci (Mutinga, 1986) [Fig. 3]. However, what one cannot assume is that, because **P. martini** is the incriminated vector in West Pokot and Baringo, it is the species of the complex likely to be the vector in the Kitui and adjacent districts. Unless it is possible to distinguish, morphologically or by other means, **P. martini** from the other two species one cannot blindly assume that **P. martini** is the vector in this area. Recently, Kaddu (1986) has consolidated

the literature on this matter and writes "P. martini has been incriminated as a vector of L. donovani on the basis of its anthropophilic habits both indoors and outdoors, their presence in endemic and epidemic foci of kala-azar, the isolation (presumably from P. martini) of parasites which cause visceral leishmaniasis in man and animals ...". He goes on to state that "The morphological similarity among female flies of the subgenus Synphlebotumus namely P. martini, P. celiae and P. vansomerenae makes it difficult to incriminate P. martini as the vector of L. donovani except in places where males of the related species have never been found." It is probably dangerous even to assume this as it may be tnat the other species are restricted to an undiscovered microhabitat or are present only under specific climatic conditions or only during certain times of the year. Mutinga & Odhiambo (1982) in a report on VL in the Machakos district found 16.4% of Sergentomyia garnhami, 12.3% of P. martini and 7.6% of S. bedfordi infected with promastigotes. They suggest that the high infection rate found in S. garnhami "strongly suggests its involvement in visceral leishmaniasis transmission" in this district. They further state that "their present findings further incriminate P. martini as the vector of kala-azar" in this area. They do not state how they could be so

sure that the **P. martini** females collected/dissected were actually that species and not either of the other two species. The other species, **Sergentomyia bedfordi** and **S. antennata**, which they have found to be infected with flagellated organisms are a species complex (see Chapter four) and neither **S. bedfordi sensu stricto** nor **S. antennata s.s.** occur in Kenya. It would appear, therefore, that the incrimination of **P. martini** as the vector of VL, in the Kitui and adjacent districts at least, is circumstantial.

The role of the three **Synphlebotomus** species in the dissemination of VL in SE Kenya is not fully understood. All three have at some stage been suggested as vectors (Lysenko, 1971; Minter, 1962; Minter & Wijers, 1963; Wijers & Minter, 1962; Wijers, 1963; Wijers & Ngoka, 1974. Wijers & Minter (1962) suggest that the only species likely to be vectors of VL are **P. martini** and **P. celiae**. Minter & Wijers (1963) suggest that **P. martini** is the principal natural vector of VL in this part of Kenya, with **P. celiae** and **P. vansomerenae** possibly involved as secondary vectors. There is no evidence, to date, that species of **Sergentomyia**, although proven vectors of lizard leishmanias, transmit mammalian leishmanias but it has been suggested (Mutinga & Ngoka, 1981) that vectors of lizard **leishmania** may play a role in the

partial immunisation against **L. donovani** infection in kala-azar endemic areas in Kenya. If this is the case the clarification of the taxonomy of the **bedfordi** complex might become important. As I have stated previously **P. bedfordi** does not occur in Kenya.

1.4 AIMS OF RESEARCH.

The correct specific identification of a vector species is important so that its geographical distribution can be established, prior to carrying out control measures. An attempt will be made to clarify the morphological identification of the species of the **Synphlebotomus** complex in East Africa (Kenya) and Southern Africa as well as those of the **Sergentomyia bedfordi** complex. Both groups have been suggested as vectors of VL and CL, and the latter, also, as possibly playing a role in conferring partial immunity on the human population at risk. Techniques used in collecting sandflies, their dissection, the subsequent culturing of parasites isolated for biochemical taxonomy and their eventual cryopreservation will be discussed. The analysis of data collected on the biology and bionomics of the Namibian vector species leads to speculation on how these factors may affect transmission of the disease in nature.

CHAPTER TWO

MATERIALS AND METHODS

2.1 FIELD TECHNIQUES.

2.1.1 Collection of sandflies.

Two methods were used for collecting sandflies.

A. Oiled cards (also called sticky papers).

This technique is a useful method to assess sandfly populations in nature. I use A4 size cartridge paper coated with castor oil (hereafter referred to as oiled cards), others use exposed X-Ray plates, plastic sheets or even lightweight A4 writing paper. The cards are prepared before going into the field using A4 sized plastic freezer containers with tight fitting lids [Fig. 5]. It is essential to pour a little oil onto each individual sheet as it is placed in the box so as to ensure that the oil soaks into every sheet. The box of cards is then left to soak either in the sun or overnight before being used.

In the field the oiled cards are removed singly and

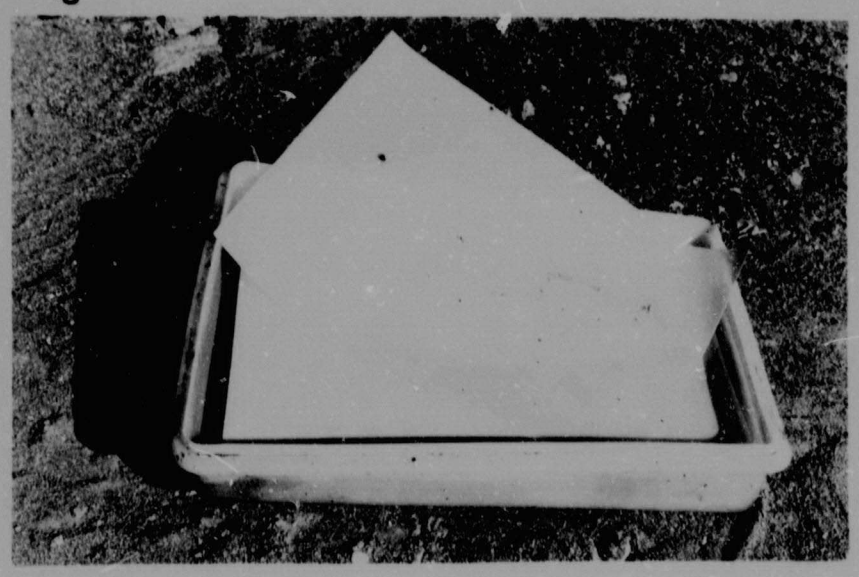
Fig. 4. Oiled cards **in situ**.

Fig. 5. A4 sized freezer container with oiled cards ready for use.

Fig. 4



Fig. 5



1) either folded into rock cracks or crevices in cliff faces and scree slopes or; 2) rolled into tubes and placed in holes under or between rocks where mammals, such as hyraxes, porcupines, rodents and mongooses live [Fig. 4], or in ground burrows of ground squirrels, mongooses, rodents and in the ventilation shafts of termite hills. Termite hills offer ideal breeding and resting sites for sandflies as the temperature and humidity within is constant and accurately maintained by the termites (Lüscher, 1961) and so provide an ideal microhabitat for sandflies. The oiled cards are placed in the late afternoon/early evening so that they lie against the walls or on the floor of the chosen site. When the flies emerge at night they stick to the oiled card. Generally, they tend to hop along the walls of the burrows rather than fly. In rocky habitats oiled cards can be left out for two or three nights but in sandy ground burrows and termite hills the oil soaks into the substrate and the cards get covered in sand so they must be visited the following morning. Trapped flies are lifted off the oiled card using a size 1 or 2 artist's brush and placed into 70% ethanol for later taxonomic study. Adding a detergent to the 70% ethanol helped to remove some of the oil that adheres to the collected flies. Used cards were disposed of by burning.

B. Light traps.

Light traps provide a means of trapping flies alive and are essential when the collected flies are to be checked for **Leishmania**, especially if live cultures are to be obtained, or where colonies of sandflies are to be set-up or maintained.

The light traps used were those of the type developed by the Arbovirus Research Unit (Jupp, 1986). They are a modification of the well known CDC light trap (as developed by Sudia & Chamberlain, 1962 of the Centres for Disease Control). Essentially the traps depend on downward suction which draws flies attracted to the light source into a collecting cage attached below a fan. Traps were powered by a Honda EM500 E portable generator. As the fan motors are 6v DC a battery charger was utilised to convert the current from 250v AC to 6v DC. From this source it was possible to run 5 to 6 traps in parallel. By varying the intensity of the light source, it was found that greater numbers of **P. rossi** species B and **Sergentomyia rima** Davidson, 1987a of the "**bedfordi** group" were attracted to a dim orange light source rather than to a brighter yellow light source. The light intensity was controlled by adjusting the amperage output of the battery charger which in effect acted as a rheostat. Certain species of sandfly including species in the **Synphlebotomus**

complex appear to be negatively phototaxic to neon and ultra-violet light sources (pers.obs.) and these were not used. Flies collected in light traps were usually in perfect condition and as they are alive, when collected, they are available for investigation as vectors of CL and VL.

2.1.2 Dissection of phlebotomines.

Microneedles for dissection were made from lengths of tungsten wire (0.05mm diam.) ±35mm long. These were dipped repeatedly in hot sodium nitrite which was melted in a crucible over a bunsen. Repeated dipping of the wire into the liquid erodes the wire at a very high temperature, producing a very fine polished point.

Phlebotomines collected in light traps are suitable for dissection. As only the females take a blood meal it was only necessary to dissect female material. Collecting cages were removed from the light traps at first light and carried to the field laboratory in a large (74 x 92cm) plastic bag. At the field laboratory the collecting cages were removed from the bag and placed in a cool place. A wad of wet cotton wool was placed on top of the collecting cage to help maintain the humidity. Flies were removed from the cages with an aspirator and blown into a glass vial (10ml McCartney

bottles were used) containing sterile saline.

Phlebotomines are covered in fine setae making them hydroscopic. It was, therefore, important to shake the vial to wet the fly as initially flies escaped when removing them from the vial prior to dissection.

A single phleobotomine was removed from the vial using the dissecting needle described above and placed in a drop of physiological saline in the centre of a glass slide. Using a WILD M5 dissecting microscope, both wings and all the legs were carefully removed and the head was severed from the body. At this stage the fine body setae have broken off and in order to minimise contamination the fly body was transferred to a second drop of sterile saline on a clean slide. Using two microneedles, one held across the abdomen just behind the thorax and the other across the intersegmental membrane of tergites 5 and 6 (i was necessary to break the intersegmental membrane between tergites 5 and 6 before stretching), and pulling in opposite directions, the microneedle manipulating the apical or genital segments drew out the entire gut [Fig. 6]. The genital segments were cut free and transferred to a drop of sterile saline on a separate slide for later dissection of the ovaries for the purpose of age grading. Enough saline was added to the gut specimen so that when a

Fig. 6. Diagram showing dissection technique used for removing the entire gut from the body of a sandfly.

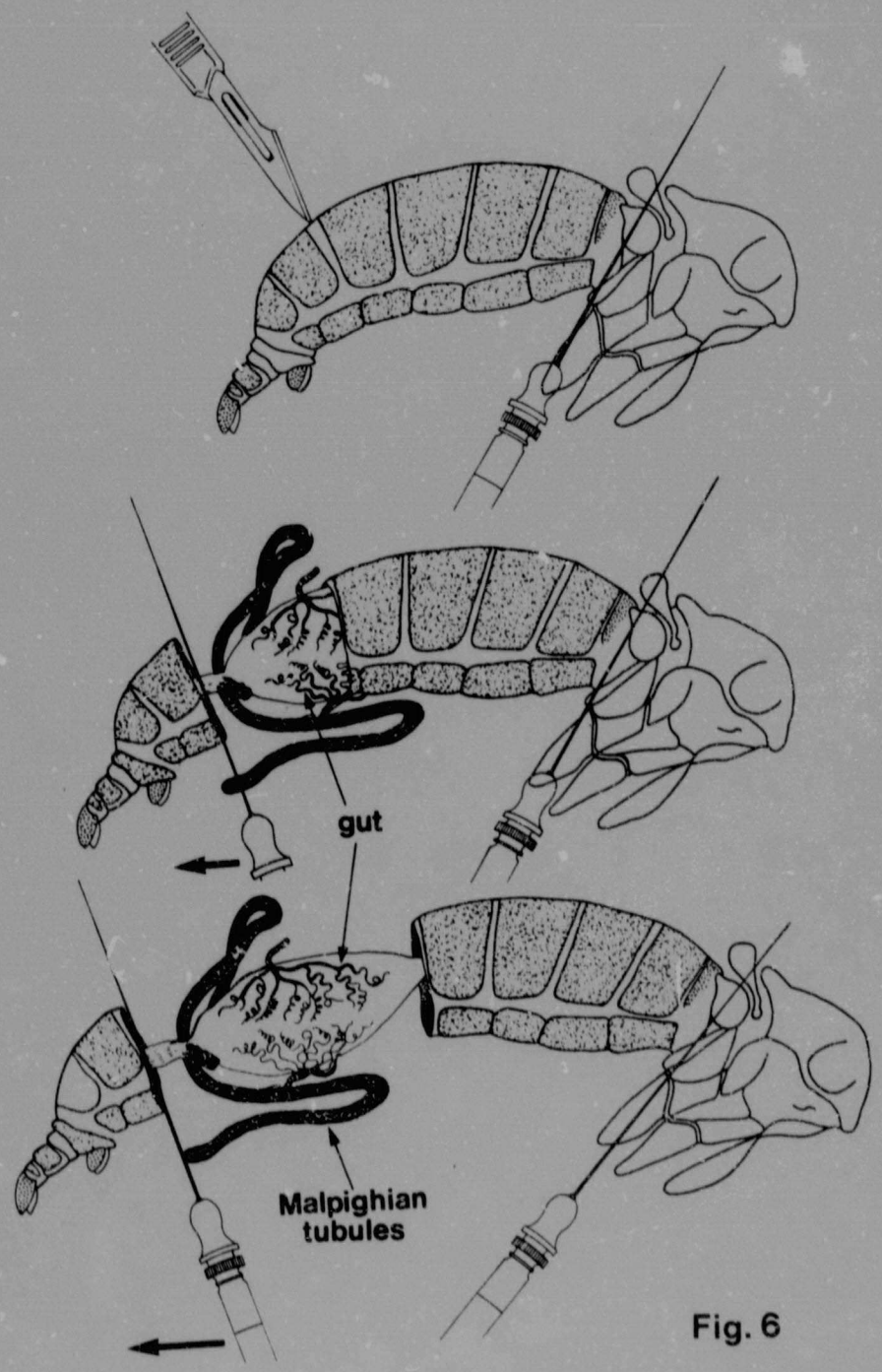


Fig. 6

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