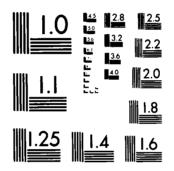
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# STUDIES OF PHLEBOTOMIN1 SAND FLIES

i.

# ANNUAL REPORT

By

D. G. YOUNG

DTIC ELECTE APR 1 4 1983

31 August 1980

Supported by

U. S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND Fort Detrick, Frederick, Maryland 21701

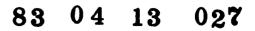
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#### ABSTRACT

- 1. Preparing Institution: University of Florida
- 2. Title of Report: Studies of phlebotomine sand flies
- 3. Principal Investigator: David G. Young, Ph.D.
- 4. No. of Pages and Date: 56; 31 Aug. 1980
- 5. Contract No. DADA 17-72-C-2139
- 6. Supported by: Dept. of the Army, Washington, D.C. 20314

A preliminary key to the phlebotomine sand flies of Kenya was prepared as an aid to identification. The need for fresh material became readily apparent. Further progress was made on illustrating important features of New World species, necessary for inclusion in a forthcoming handbook of the American sand flies. New geographic records and specimens for the reference collection were given to the Principal Investigator by colleagues working in Guatemala, Mexico and Brazil. Several descriptive papers were submitted for publication and another, a review of a new species group (Microps Group), was nearly finished. Attempts to rear and maintain three U.S. phlebotomine species were successful. A key problem of high larval mortality faced by many investigators was overcome by the development of a simple diet discussed in a short note submitted for publication,

iii

7. Key Words: Sand fly

Lutzomyia

Phlebotominae

Phlebotomus

Leishmaniasis

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## PROGRESS REPORT DADA 17-72-C-2139

#### Introduction

The importance of phlebotomine sand flies as vectors of leishmaniasis and arboviruses in many areas of the world is considerable. The W.H.O. scientific working group on leishmaniasis (1977 report) stated that ". . . taxonomic studies of these vectors are of fundamental importance due to the difficulty in identifying them." Recommendations of this group included continued maintenance of phlebotomine reference collections and support for research programs dealing with systematics of these insects. The group stressed the "need for new practical field keys" in some areas.

#### Objectives

The objectives under this contract are similar to those outlined by the W.H.O. working group. In addition to studying the American species (the projected date of completion of a handbook on these is June, 1982), we began to study the phlebotomines of Africa, beginning with Kenya, and the Middle East. Objectives include:

- Preparing keys, illustrations, and other aids to identification both by geographic areas and by taxonomic groups.
- Arriving at a more satisfactory classification of the subfamily phlebotominae.
- 3. Building a reference collection on a worldwide basis.
- 4. Maintaining one or more species in laboratory colonies to provide

immature stages and adults necessary for the evaluation of nonmorphological taxonomic techniques.

#### Results

Slide mounted specimens of Kenyan phlebotomines (27 species) in our collection enabled the Principal Investigator and Capt. Raymond Beach to compile a preliminary identification key to the species known to occur there (Appendix I). The specimens on hand were poorly mounted over 20 years ago in temporary medium; therefore, it was not possible to critically evaluate all of the taxonomic characters believed to be important. A forthcoming trip to that country during June-July, 1981, working in cooperation with investigators at the International Centre of Insect Physiology and Ecology, Nairobi, as an invited scientist and WRAIR should be fruitful in terms of obtaining adequate samples of various species. One of the unsolved taxonomic problems of direct importance to the epidemiology of visceral leishmaniasis in Kenya is the apparent impossibility of separating the 3 *Synphlebotomus* spp. females by conventional means. One of these, *Phlebotomus martini* is the suspected vector but *P. celiae* may also be involved.

Work continued on the handbook of the New World species. The emphasis is being placed on identification; thus, many illustrations will be included--a time consuming but necessary aspect of the project. Most of the time spent on this handbook during the past year involved making drawings.

Colleagues sent specimens to the PI from Mexico, Guatemala, Brazil and the U.S.A. for determination. About 1000 flies were slide mounted and identified; those from Mexico and Guatemala, both little-collected areas, were especially interesting. Dr. Charles Porter, CDC medical entomologist in Guatemala, collected 9 species previously unknown in the Republic. These are Brumptomyia hamata, B. galindoi, Lutzomyia odax, L. ovallesi, L. carpenteri, L. shannoni, L. texana, L. trinidadensis and L. panamensis. The latter species is an incriminated vector of cutaneous leishmaniasis in Panama.

Mexican material, collected at Miacatlán (Morelos) by Dr. Marco Camino and sent to the PI, is valuable because this locality is close to the type locality of 3 poorly known taxa--L. durani, L. hardisoni and L. dodgei. These species and at least 7 others, including the common L. longipalpis, were added to our reference collection. Information on these species will be included in the handbook.

Descriptions of two Amazonian species were sent to press (Appendices II and III). One of these forms, *L. olmeca nociva*--a man biter, belongs in the *flaviscutellata* complex which includes all the known vectors of *Leishmania mexicana* from Mexico to Brazil. The review of the *Lutzomyia davisi* complex should be published by April, 1981. A paper on a new group, the *Microps* group with 5 South American Species, was nearly completed.

Lutzomyia shannoni, a man-biting species in parts of its widespread geographic range (U.S.A.-Argentina), was collected for the first time in South Carolina and at Ft. Bragg, North Carolina, by LTC Ron Intermill in 1980. The identifications were made by the PI and CPT Peter Perkins, Ph.D. graduate student working with the PI [the fact that shannoni occurs at Ft. Bragg where Leishmania-infected soldiers apparently live and train indicates that transmission from vector to man or other mammal could occur]. The recent identification of Leishmania infantum, an Old World visceral

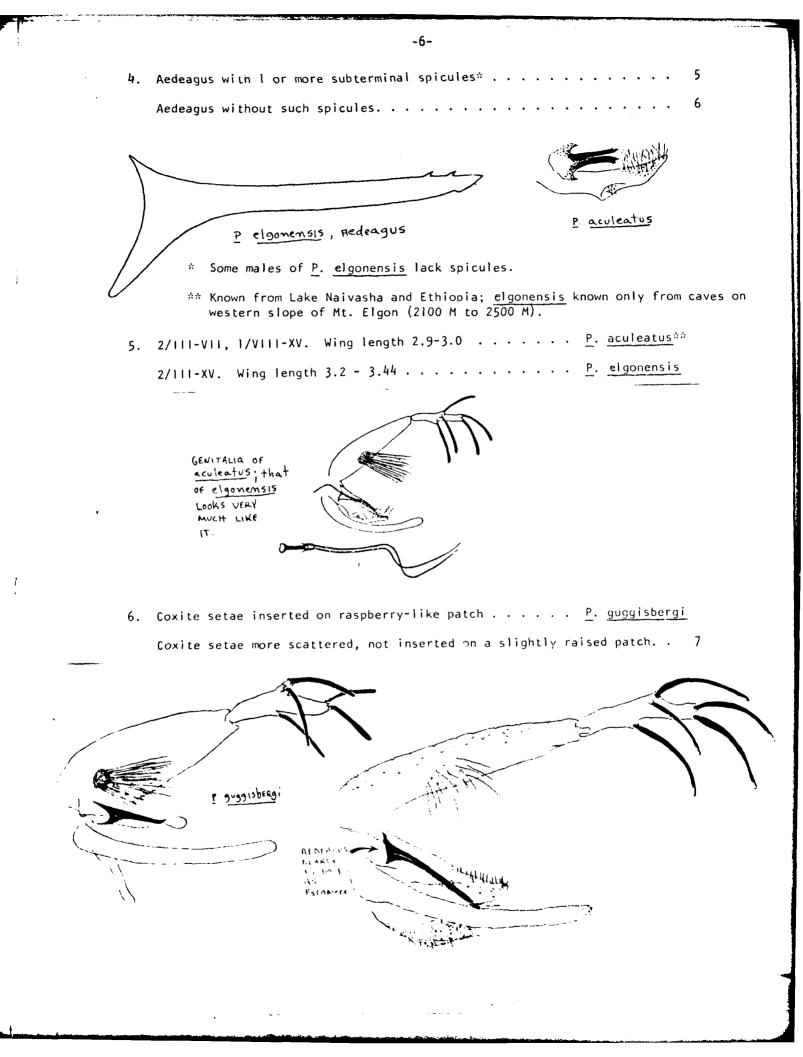
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Leishmania, in Oklahoma in dogs (LTC Larry Hendricks, personal comm.), indicates that the successful establishment of an imported infection in the U.S.A. is possible. L. shannoni and two other U.S. species were successfully reared and colonized in our laboratory, mainly by graduate students working with the PI. Better methods of rearing resulted in a scientific note which was submitted to the <u>Journal of Medical Entomology</u> in 1980 (Appendix IV). The importance of rearing these insects in relation to taxonomy is clearly evident when morphologically inseparable, sibling species are considered. These methods for rearing immatures and handling adults of the *Symphlebotomus* complex in Kenya will be tried. If successful, then males and females in this complex can be properly associated, and identified specimens can be studied by various techniques, including cuticular hydrocarbon analysis.

Specimens of *L. anthophora* were sent to the Department of Arboviral Entomology, Ft. Detrick, for subcolonization and subsequent vector competence studies. The importance of maintaining phlebotomine colonies, especially vectors, is considerable--not only for taxonomic studies but also for experimental work not covered under this contract.

-4-

# Appendix I PRELIMINARY KEY TO THE PHLEBOTOMINAE OF KENYA Males 2 10 NOTE STRONG SPINE ON LOWER ARM OF PARAMERE P. neischi P. rodhaini 2. Paramere bilobed, i.e. forked. Pleural setae present..... S. heischi<sup>#</sup> Paramere simple. Pleural setae absent. (Genus Phlebotomus, in part). . 3 3. Genital filaments 3x to 11x length of pump. Persistent hairs inserted directly on coxite, i.e. no arm or tubercle. (Subgenus Larroussius) . . . . Genital filaments shorter than 3x length of pump. Coxite tuft of persistent hairs inserted on long arm. (Subgenus Synphlebotomus) . . . 8 SEE COUPLET & OF FEMALE KEY FOR FIGURE OF PLEURAL SETAE. -5-



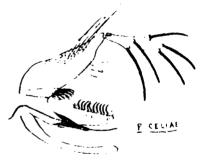
7. Aedeagus nearly as long as parameres, its tip straight or nearly so. Aedeagus shorter than parameres, its tip turned upwards . . . <u>P. pedifer</u> Aedeagus Shorter than parameres, its tip turned upwards . . . <u>P. pedifer</u> Aedeagus Shorter than parameres, its tip turned upwards . . . <u>P. pedifer</u> Aedeagus Shorter than parameres, its tip turned upwards . . . <u>P. pedifer</u> Aedeagus Shorter than parameres, its tip turned upwards . . . <u>P. pedifer</u>

-1-

Coxite setae of 7 or so spatulate hairs and some thin ones. Parameres with dorsal sickle-shaped setae.
 Coxite and paramere setae simple
 Source and paramere setae simple

Pcellae

TIP

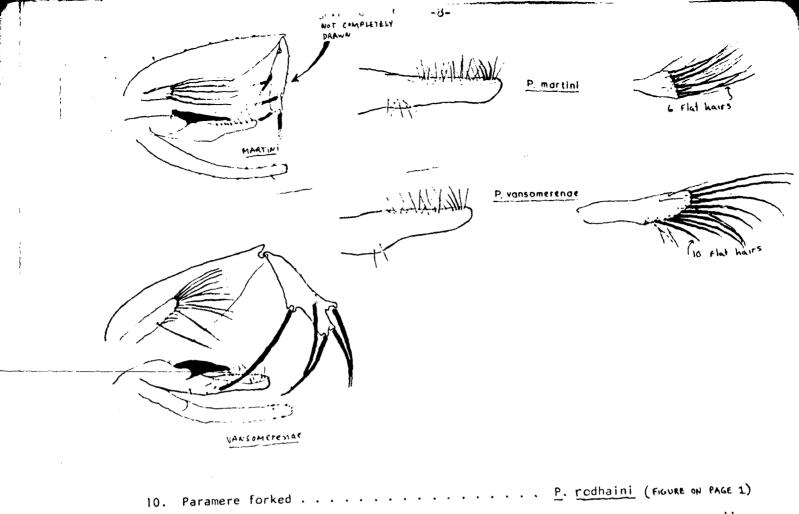


PARANERE

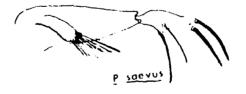


- 9. Coxite tuft of 6 flat hairs and about 12 thin ones. . . . <u>P. martini</u> Coxite tuft of 10 flat hairs and fewer than 12 thin ones . . . . . . . . . . . . . . . <u>P. vansomerenae</u>
- \* Vector of Kala-azar in Sudan. Also called p-orientalis by various autions \*\* Vector of cutaneous Leishmaniasis in Kenya.

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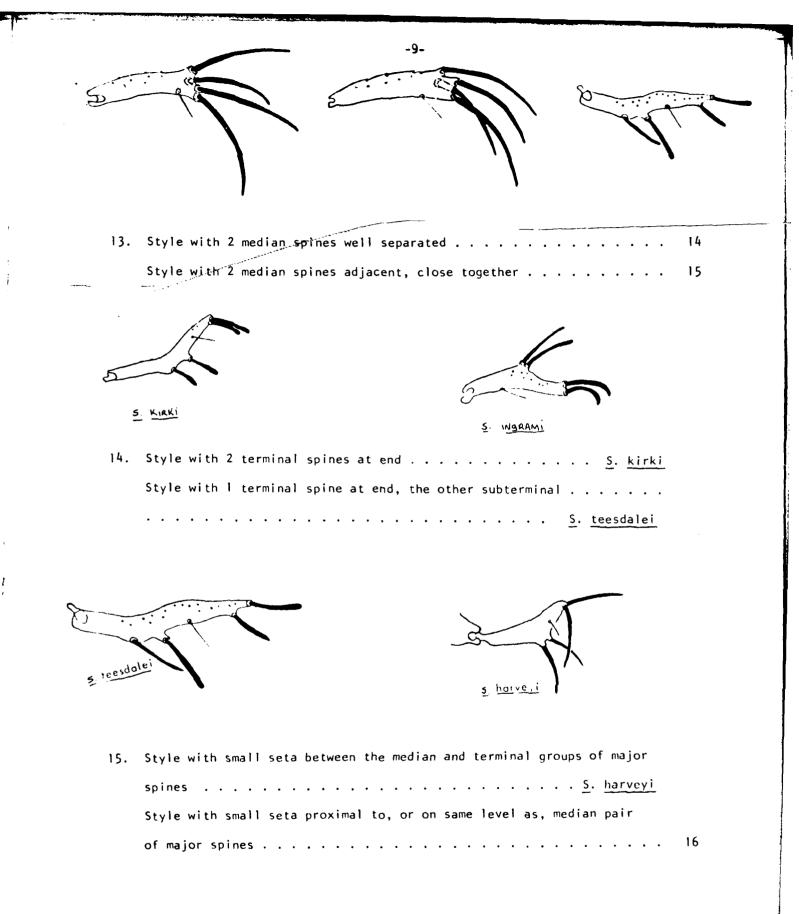


- 11. Coxite with persistent setae inserted on a long arm . . . P. <u>saevus</u> Coxite with persistent setae usually absent but, if present, not inserted on an arm or tubercle. (Genus <u>Sergentomyia</u>, in part). . . . . 12



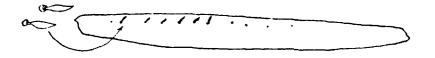
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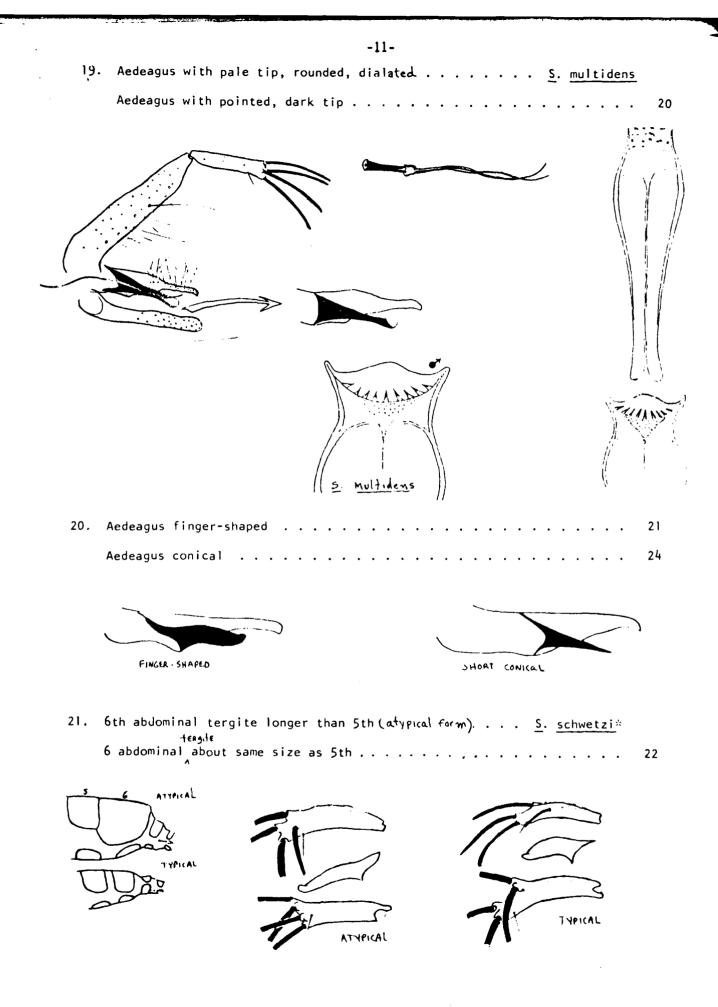


16. Cibarium with ca. 35 straight pointed teeth arranged in an arc; vertical teeth punctiform in 2-3 rows . . . . . . . . . . . . S. serrata Cibarium with ca. 30 very short teeth; few or no punctiform vertical teeth . . . 17. Style with 2 terminal spines and 2 others at distal 3/4 . . . . . . ••••• S. <u>schwetzi</u> (typical form) Style with all spines terminal or subterminal, i.e. close together. . . 18 schwetzi (also see Figures below couplet 21) P SERRATA OT Con communa AFFINIS AFFINIS 5. affinis VORAK 8 AFFINIS VORA

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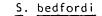


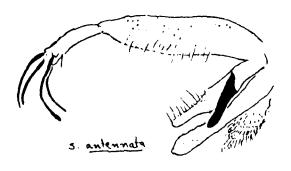
22. Paramere (0.16 mm long). About as long as lateral lobe . . . S. yusafi ( constant areas) 23 

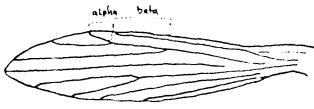


23. Antennal segment 3 short (.095-.100); alar index a/b = 0.50-0.57; pigment patch roughly circular. Palpal segment 4 = to or but little Antennal segment 3 longer (.100-.150); alar index 0.64; pigment patch short and broad. Palpal segment 4 clearly longer than 3. . . . . . . . . . . .

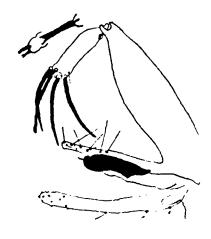
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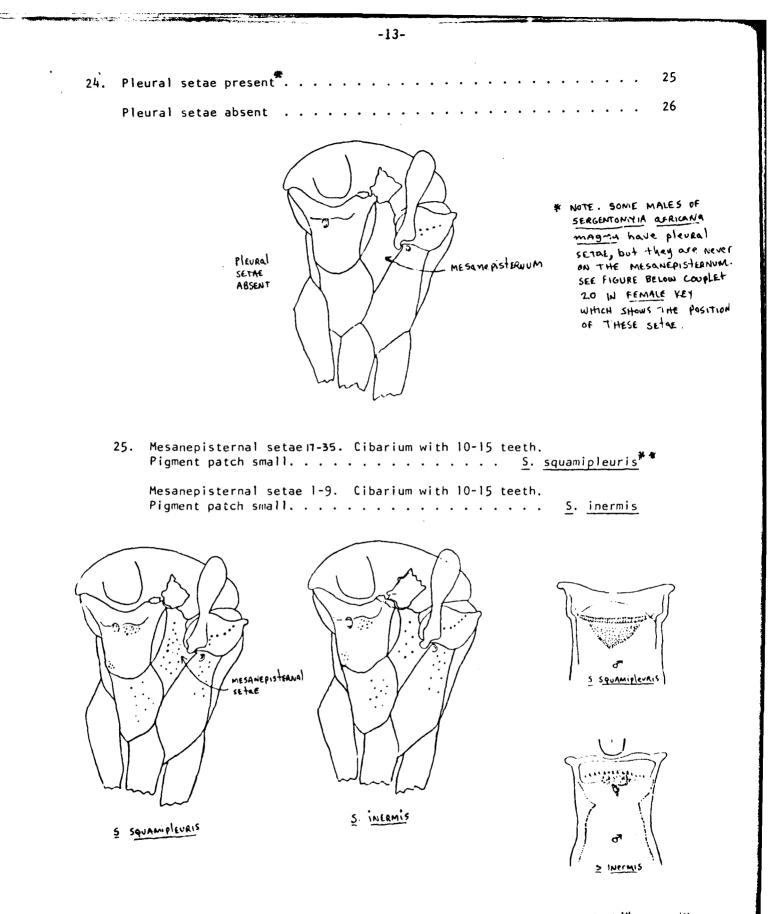


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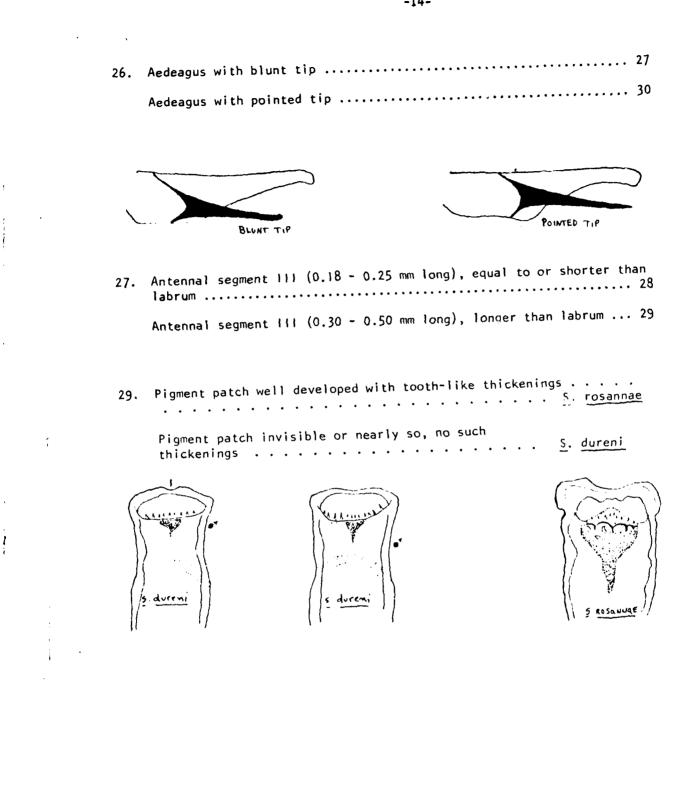
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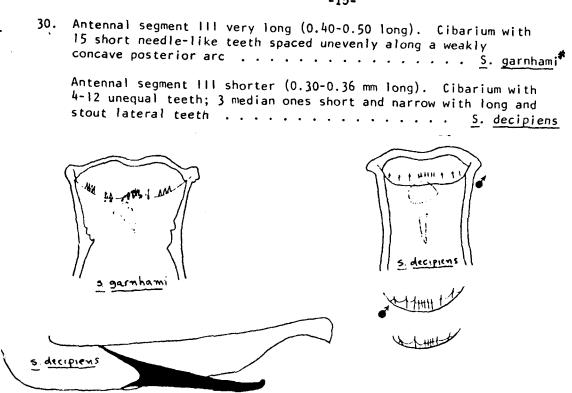
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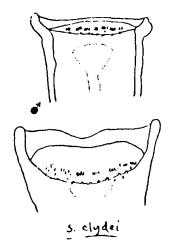
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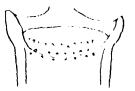
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Cibarium with 10-12 distinct teeth and 2-3 rows of anterior punctiform denticles. Tergites 5 and 6 subequal in size ...... S. adleri



1



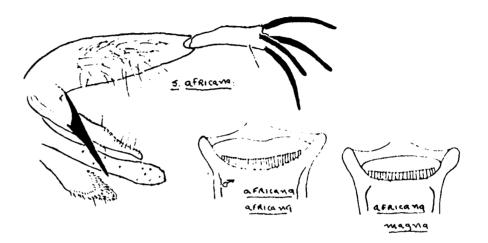
5. adleri

\* civilei sometimes bites MAN, As does garnhami

-15-

32. Antennal segment III < 0.28 mm. No pigment plate. Coxite with numerous median and distal setae. Pleural setae present or not .. 33 Antennal segment III > 0.28 mm. Pigment plate present. Coxite

 Pleural setae usually present (see figure below; couplet 20 in female key). Cibarium with 14-23 teeth ..... S. africana magna



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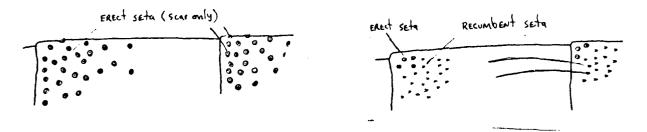
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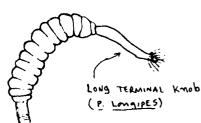
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-16-

#### Females

- Cibarium without teeth or with only tiny spicules. Pigment patch absent. Hind ends of abdominal tergites 2-6 with erect hairs. Pleural setae absent. (Genus <u>Phlebotomus</u>) . . . .







P. elgonensis

2

Wing length 1.9-2.5.	Antennal segment 3,0.25-0.30 mm
• • • • • • • • • • •	• • • • • • • • <u>P. langeroni</u> orientalis
	(= orientalis of various authors)

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\* FEMALES HAVE BEEN REPORTED BITING MAN

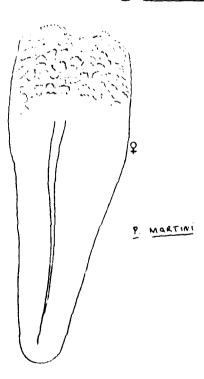
-18-

5. Spermathecae with 4-6 segments. Pharynx with armature of coarse teeth. (Subgenus <u>Paraphlebotomus</u>) . . . . . <u>P. saevus</u>

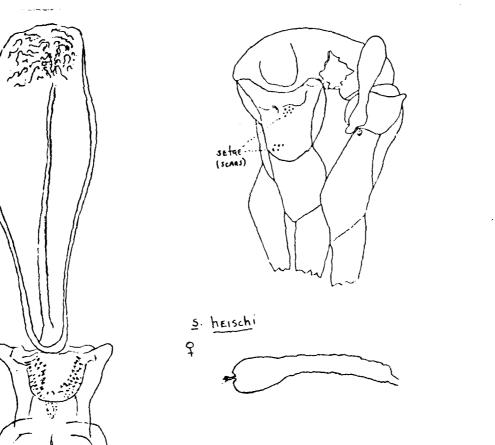
Spermathecae with	9-10 segments. Pharynx with ridges	
and small teeth.	(Subgenus Synphlebotomus)	P. martinl
		<u>P. celiae</u>
	P. 1	ansomerenae



P SAEVUS



6. Cibarium with lateral longitudinal and irregular rows of teeth. Pleural setae positioned as shown. (Subgenus <u>Parvidens</u>). **\*** 



. . . . . . .

Spermathecae otherwise, with no setae; pleural setae absent. . . 9



5 SQUAMIPLEVRIS

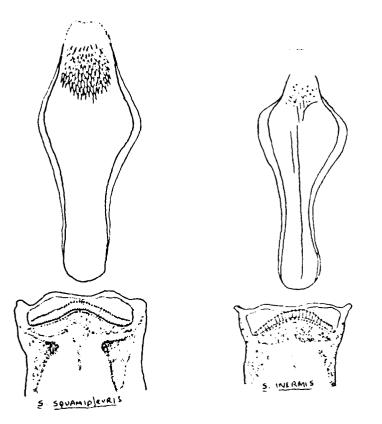
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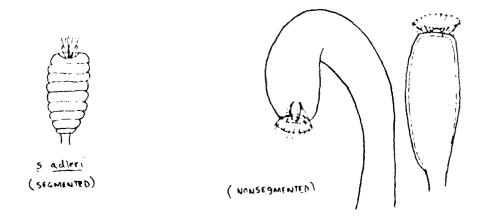
GRASSOMYIQ - TYPE SPERMATHE CQ \* SERGENTOMYIA AFRICANA MAZMA (COUPLET 20) MAY Also have ONE GROUP OF PLEURAL SETAE BUT THE SPERMATHECAE ARE FUITE DIFFERENT.

A RARE MAN- WHER

-19-

-20-





Front and hind femora with a row of conspicuous dark spines (see of wey). 10. Pharynx with about 50 teeth with long points; cibarium with TITT HUMAN s adleri Constanting the second 1 . . 5. adleri AFFINIS ACFINIS AFFINIS ( VOIAX FORM

1

3 AFFINIS VORAX ALSO OCCURS IN KENNA THE FEMALES OF THIS FORM HAVE GO OR MORE HORIZONTAL TEETH IN THE CLOAFIUM RATHER THAN 34 TO 40 FOR 5 AFFINIS AFFINIS.

, ...

-21-

- -22-12. Cibarium with well developed row of teeth; no vertical LLLKAHLLA W. LUTHIN IN THIN WANNAN THIN ++++++5. clydei S. clydei graingeri 13. Cibarium with 14-16 stout, pointed teeth; pigment patch large S. meilloni Cibarium with 25-27 teeth, the lateral ones larger and farther apart than the median teeth . . . . . . . S. suberecta - --- - -A MALIJUNIA UNIA 1111 s subfrecta S. MEILLON' Cibarium with 25 or more horizontal teeth. Pigment patch in the 14. form of a pointed helmet .... S. graingeri Cibarium with 20 or fewer horizontal teeth. . . . . . S. clydei 15. Pharynx armed with numerous teeth in highly packed rows. . . . . 16 Pharynx unarmed or with only a few scattered distinct teeth . . . '9 416414 PALKED RIDLES &
  - A FFW SCATERED TEETH / types of affrong

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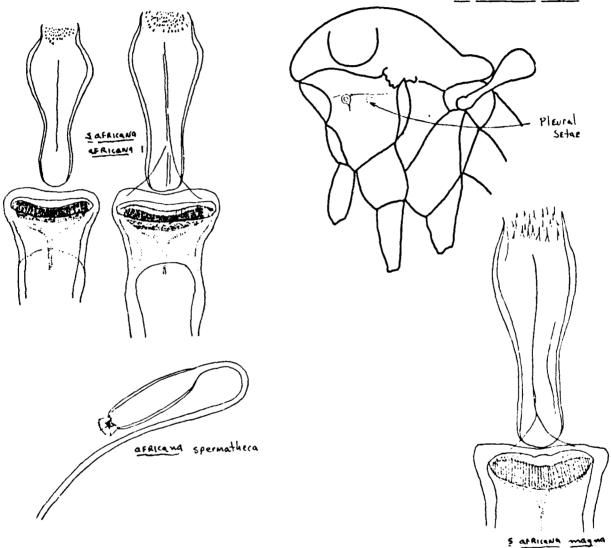
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. 16.	Cibarial teeth suboval, monomorphic
	Cibarial teeth in middle smaller than lateral teeth
17.	Antennal segment III < .100 mm; palpal segment 4 somewhat > or = 3
	Antennal segment III > .100 mm (coastal sp. ) . <u>S. yusafi</u> (rare)
	Santennata Einche FORM)
	<u>S. bedroed</u> i bedroedi <u>S. visari</u>
	Cibarium with 26-28 <u>small</u> pointed teeth, the median ones smaller than laterals <u>S. gracilis</u> (rare)
	Cibarium with 24-28 teeth on an arc, the 12-13 median teeth small, the lateral teeth stout, palpal segment 4 clearly $> 3$
* <u>S</u> . <u>b</u>	edfordi w/ indented pharyngeal wall (compare Quate,Fig.19 B & 20C)
99 10- 10-	Himmententitient Himmenten Wall, Typical badroadi But Not autennat S geacilis S geacilis S geacilis S geacilis

19. Pharynx armed with a small number of scattered denticles ...... 20

Pharynx unarmed or with fine folds, ridges, or broad scales having finely denticulate (dot-like) posterior borders ...... 21

20. Gbarium armed with about 60 teeth; pigment plate black, often obscuring teeth ..... <u>S. africana africana</u>

Gbarium armed with not more than 45 teeth. Pigment patch usually brown; 1-6 insertions (ie. setal scars) on thoracic pleurae ..... S. africana magna



\* Abonnenc (1972) elevates this subspecies to species rank, calling it S. magna. Not all specimens have pleural setae in Ethiopia. Specimens in Kenya may or may not show this variation. It is not clear whether one or both of the forms in couplet 20 occurs in Kenya.

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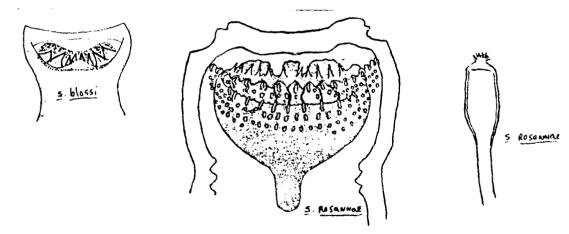
-24-

21.	Antennal segment III very long (.39044 mm)
	Antennal segment III long (.09~.38 mm)
22.	Cibarium with 11-12 teeth and only 1 row of anterior vertical teeth
	Cibarium armed with 16 strong teeth and w/ 6-8 rows of anterior vertical teeth



5 Multideus

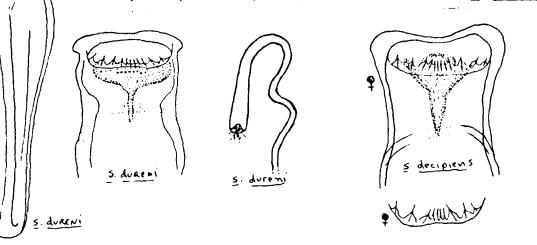
- 25. 8 cibarial teeth, the 4 median ones short and narrow, the lateral teeth stout and wide pigmented plate bilobed. S. blossi



26. Cibarium armed w/ 10-12 short retractile teeth, scarcely visible; very stout, rounded tooth-like folds present anterior to the cibarial teeth; 3-6 rows of anterior vertical teeth. Pigmented plate large and massive . . . . . . . . . . . . S. rosannae

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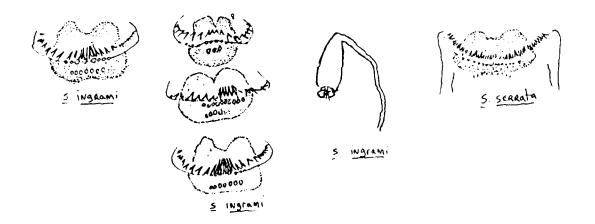
Cibarium armed with 11-15 teeth; the laterals stouter than the median teeth; pigmented plate in the shape of a pointed helmet, w/ a posterior prolongation . . . . . . . . <u>S</u>. decipiens



28. Cibarium armed with 24-28 polymorphic teeth, 2 or 3 rows of anterior vertical teeth, of which the central teeth are noticeably larger; a few erect hairs on abd. tergites 2-6....S. ingrami

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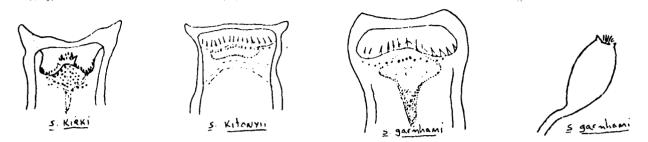
Cibarium with 40-52 teeth and 3 rows of subequal vertical teeth. No erect hairs on abdominal tergites 2-6. . . S. serrata



29. Cibarium with 1 or several rows of vertical teeth, or an<br/>unorganized group of teeth30Cibarium without vertical teeth32

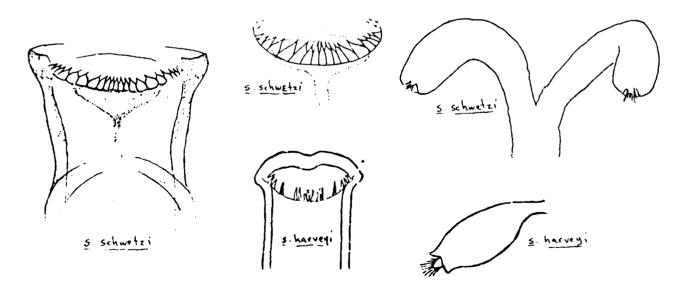
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-26-



31. Pigment patch heart-shaped; antennal segment 3 0.26 - 0.32 mm long; cibarium with 15 long subequal needle-like teeth on a slightly sigmoidal. A row of anterior vertical teeth.
S. kitonyii

32. Spermatheca tubular with the terminal knob embedded in the body. Cibarium with 16-18 teeth. . . . . . . . <u>S. schwetzi</u>\*



\* Sometimes bites MAN. THE ATYPICAL AND TYPICAL FORMS ARE SEPARAted only in the

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#### Appendix II

Sand Flies of the Central Amazon of Brazil. 2. Description of Lutzomyia (Trichophoromyia) ruii n. sp. (Diptera: Psychodidae)<sup>1</sup>

Jorge R. Arias<sup>2</sup> David G. Young<sup>3</sup>

#### Abstract

Lutzomyia (Trichophoromyia) ruii Arias & Young n. sp. is described and illustrated from both sexes commonly found near Manaus, Brazil. Females are not anthropophilic. Information on seasonal distribution is given. Lutzomyia (Trichophoromyia) melloi (Causey & Damasceno) is a junior synonym of Lutzomyia (T.) inini (Floch & Abonnenc) (NEW SYNONYM).

#### Introduction

Sherlock and Guitton (1970) reviewed the phlebotomine subgenus <u>Trichophoromyia</u> Barretto, 1962, described a new species, and provided a key to the males of 18 species. Two of them, <u>Lutzomyia</u> (<u>T</u>.) <u>melloi</u> (Causey & Damasceno, 1945) and <u>Lutzomyia</u> (<u>T</u>.) <u>inini</u> (Floch & Abonnenc, 1943) were separated in couplet 4 by genitalic characters. We now believe, however, that <u>L</u>. <u>inini</u> is a senior synonym of <u>L</u>. <u>melloi</u> based on recent determinations of males from Brazil and French Guiana by us and M. Emile Abonnenc (NEW SYNONYM). Recently, Young (1979) described 3 new <u>Tricho-</u> <u>phoromyia</u> spp. from southern Colombia. Here, we describe another species which is rather common in the vicinity of Manaus, Brazil.

Lutzomyia ruii is named in honor of Mr. Rui Alves de Freitas of the Instituto Nacional de Pesquisas da Amazônia for his dedicated service in the continuing study of phlebotomines and leishmaniasis in the Amazon Basin of Brazil. Terminology follows that of Young (1979). The description is based on the holotype and allotype with ranges of some measurements of paratypes  $(111\sigma\sigma, 1199)$  given in parentheses. Measurements are in millimeters.

Lutzomyia (Irichophoromyia) ruii Arías & Young, n. sp.

### Fig. 1-9

Male:

A large dark sand fly; pleura moderately pigmented, mesonotum strongly infuscated. Wing length 2.10 (2.06-2.18); width 0.56 (0.53-0.56). Cibarium with about 12, scattered dot-like remnants of vertical teeth; cibarial arch distinct only at sides, without pigment patch. Pharynx 0.18, unarmed. Head height ; width

. Eyes large, separated by 0.126 or by distance equal to 7.1 facet diameters. Flagellomere I, 0.23 (0.21-0.24), combined length of II + III slightly longer than I; ascoids with very short distinct posterior spurs, the distal tips of ascoids on II reaching beyond flagellomere, on all flagellomeres except last 3. Labrum 0.23 (0.21-0.24) long. Length of palpal segments (holotype): 1 (0.04), 2 (0.10), 3 (0.40), 4 (0.06), 5 (0.14); palpal sensillaat end of segment 2 and along middle third of 3. Pleura with 15 (7-15) upper and 2 (2-4) lower episternal setae. Length of wing vein sections: alpha 0.56 (0.49-0.58), beta 0.28 (0.26-0.33), delta 0.42 (0.28-0.42), gamma 0.22 (0.22-0.28). Length of femora, tibiae and basitarsi of holotype: foreleg, 0.86, 1.13, 0.68; midleg, 0.79, 1.35, 0.83; hind leg, 0.86, 1.58, 0.90. Genitalia: style 0.23 long, with 4 major spines arranged as shown, no subterminal seta. Coxite ca. 0.39 long x 0.12 wide, bearing a median group of ca. 45 long slender hairs. Paramere simple as shown. Aedeagus broad, well pigmented. Genital pump 0.18 (0.15-0.18)

long, each filament 0.87 (0.87-1.02) long or  $4.8 \times$  length of pump, tip simple. Lateral lobe 0.38 (0.38-0.40) long. Cercus as shown.

Female: Wing length 2.20 (2.20-2.40); width 0.64 (0.64-0.75). Cibarium, with 10-12 slender pointed, more or less equidistant horizontal teeth and 20-30 vertical teeth, median teeth larger than others; cibarial arch complete; pigment patch well infuscated. Pharynx 0.19 (0.18-0.20) long, unarmed. Head height : width Eyes large, separated by 0.55 or by distance equal to 7.2 facet diameters. Flagellomere I 0.20 (0.20-0.25) long, II + III slightly longer than I; ascoids as in male, on all flagellomeres except last 3. Labrum length 0.34 (0.34-0.43). Length of palpal segments of allotype: 1 (0.05), 2 (0.15), 3 (0.19), 4 (0.06), 5 (0.14); palpal sensilla as for male. Pleura with 9 (9-17) upper and 2 (2-4) lower episternal setae. Length of wing vein sections: alpha 0.70 (0.64-0.78), beta 0.27 (0.27-0.30), delta 0.49 (0.41-0.55), gamma 0.25 (0.20-0.29). Length of femora, tibiae and basitarsi of allotype: foreleg, 0.75, 1.01, 0.71; midleg, 0.75, 1.24, 0.83; hindleg, 0.83, 1.46, 0.94. Spermathecae annulated except for smooth subterminal portion; individual ducts very long, ca. 6.5 x length of spermatheca; common duct shorter than spermatheca.

Type Data: Holotype male (DCDC-419, no. 1).

Reserva Ducke, 26 km E of Manaus, Amazonas, Brazil; light trap; 6 Dec. 1977; J. Arias, R. Freitas & J. Vidal colls. <u>Allotype</u> female (Ducke-CDC, no. 2). Same data but taken on 18 June 1976. <u>Paratypes</u> (120mm, 3099). Reserva Ducke, Reserva Campinas (43 km NE of Manaus), at km 30 and 4 km S of km 56 Rodovia Torquato Tapajós-

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Estrada AM-010; CDC light traps, flight traps, soil emergence traps, and animal burrows; 1974 to 1979, J. Arias et al. colls.

Holotype, allotype and 20 paratypes to be deposited at Instituto Nacional de Pesquisas da Amazonia, Manaus. Other paratypes in Brazilian collections at Universidad Federal de Minas Gerais and São Paulo; Instituto Oswaldo Cruz, Rio de Janeiro; Instituto Evandro Chagas, Belém; Museu Paraense Emilio Goeldi, Belém. Remaining paratypes in collections of U.S. National Museum (Natural History), Washington; Florida State Collection of Arthropods, Gainesville, Florida; Instituto de Salude Publica, Lima; British Museum (Natural History), London. All specimens slide mounted.

Discussion:

We associated the sexes of <u>L</u>. <u>ruii</u> on the basis of collecting data. No other <u>Trichophoromyia</u> spp. were taken at the study site 30 km NE of Manaus during a 62 week period of time.

This species occurs with <u>Lutzomyia</u> (<u>Trichophoromyia</u>) <u>ubiqui-</u> <u>talis</u> at Reserva Campinas. The males differ readily by the shape of the parameres (more slender in <u>ubiquitalis</u>) and by the length of the genital filaments (shorter than 4X length of pump for <u>ubiquitalis</u>; over 4.5X for <u>ruii</u>).

The females of <u>ruii</u> and <u>ubiquitalis</u> closely resemble each other in nonsexual traits of color, cibarial armature and wing venation (This generally is true for the other <u>Trichophoromyia</u> females as well ). However, the shorter sperm ducts and larger terminal knob of the spermathecae of <u>ubiquitalis</u> (Fig. 10) easily serve to distinguish the females. Floch and Abonnenc (1943, 1952)

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describe and illustrate the <u>ubiquitalis</u> female as <u>Phlebotomus</u> <u>cauchensis</u>, an accepted junior synonym.

From the <u>Trichophoromyia</u> males having a median tuft of numerous coxite setae such as <u>L. inini</u>, <u>L. auraensis</u> (Mangabeira, 1942) and <u>L. loretonensis</u> (Llanos, 1964), <u>L. ruii</u> differs in the shape of the parameres (see Sherlock & Guitton, 1970, for figures of these other males).

Light trap catches from the site, 30 km NE of Manaus, during a 62 week period (Fig. 11) indicated population peaks of <u>L</u>. <u>ruii</u> adults from August-November, 1977 and February-March and August, 1978. Females do not attack man and their preferred hosts remain unknown.

We wish to thank M. Emile Abonnenc for identifying a male of <u>L</u>. <u>inini</u> collected in French Guiana by Dr. T.H.G. Aitken. Also, we appreciate the valuable assistance of Mr. João Ferreira Vidal in the field and laboratory.

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- Sherlock, I.A. & N. Guitton. 1970. Notas sòbre o subgènero <u>Trichophoro-myia</u> Barretto, 1961 (Diptera, Psychodidae, Phlebotominae). Rev. Brasil. Biol. 30:137-150.

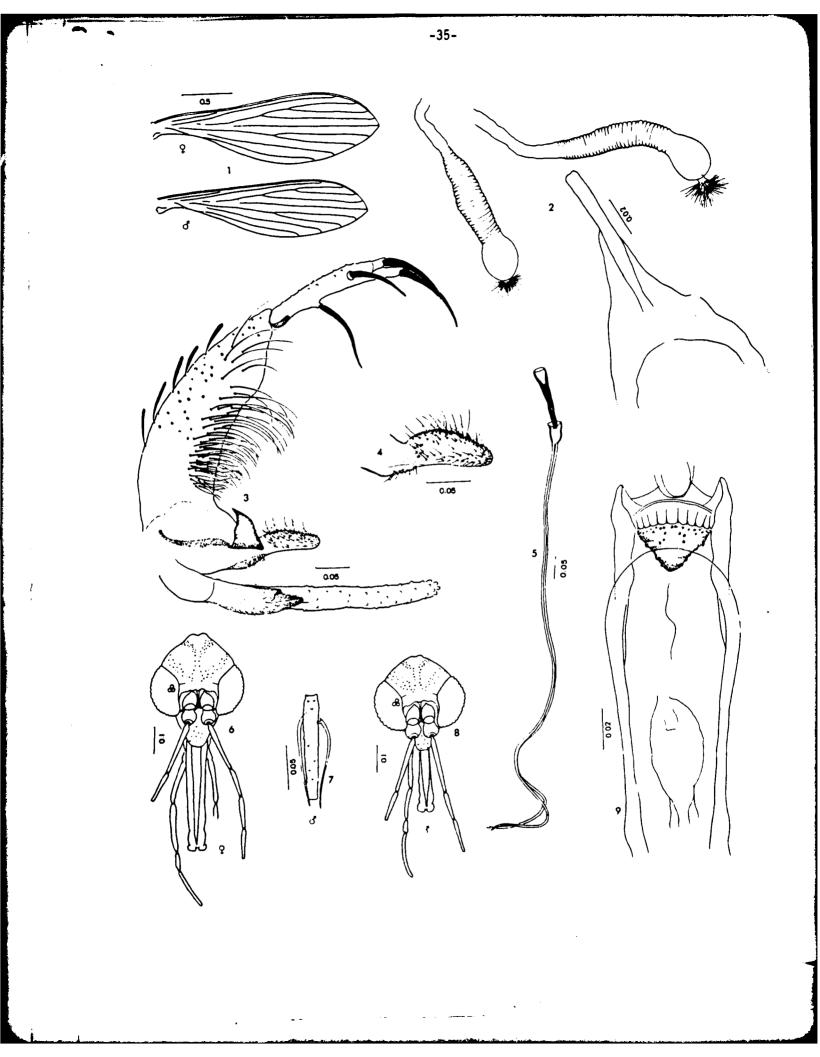
## Footnotes

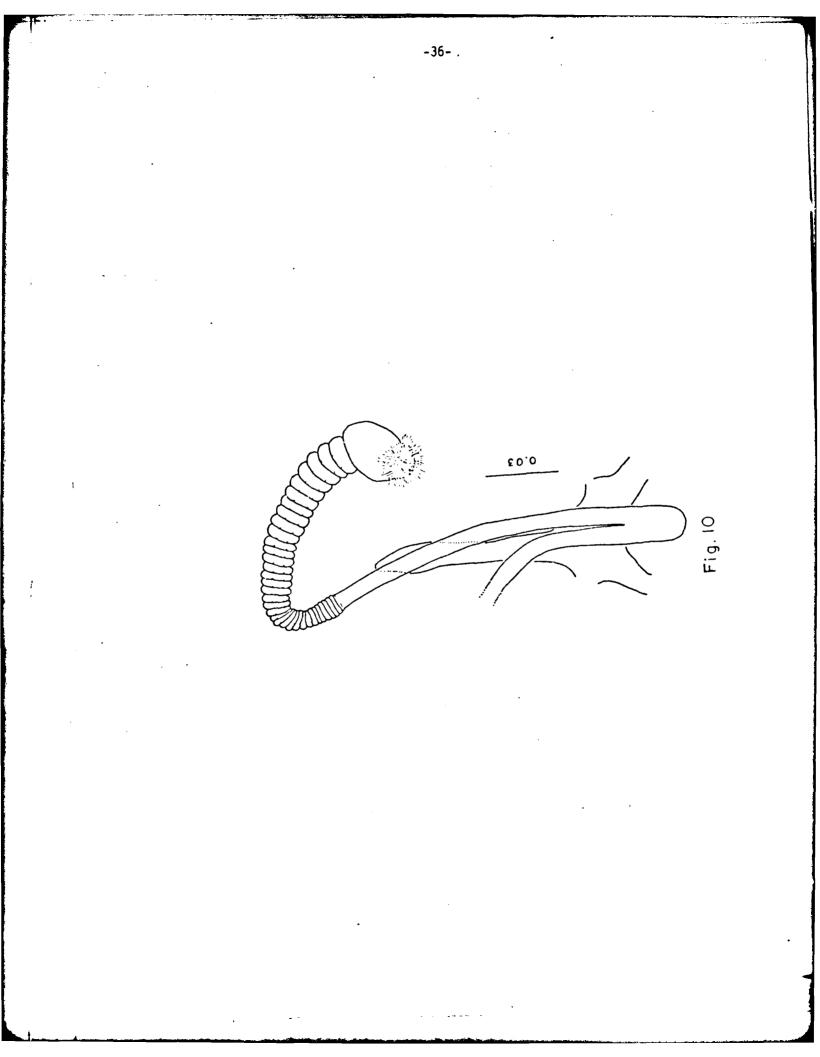
- Research was partly funded by CNPq Grant SIP 08/131; INPA Project 2017/103, and U.S. Army Research and Development Contract DADA 17-72-C-2139.
- 2. C.P. 478, Manaus, 69000, Amazonas, Brasil (Address for reprint requests).
- Dept. of Entomology and Nematology, University of Florida, Gainesville, Florida U.S.A. 32611.

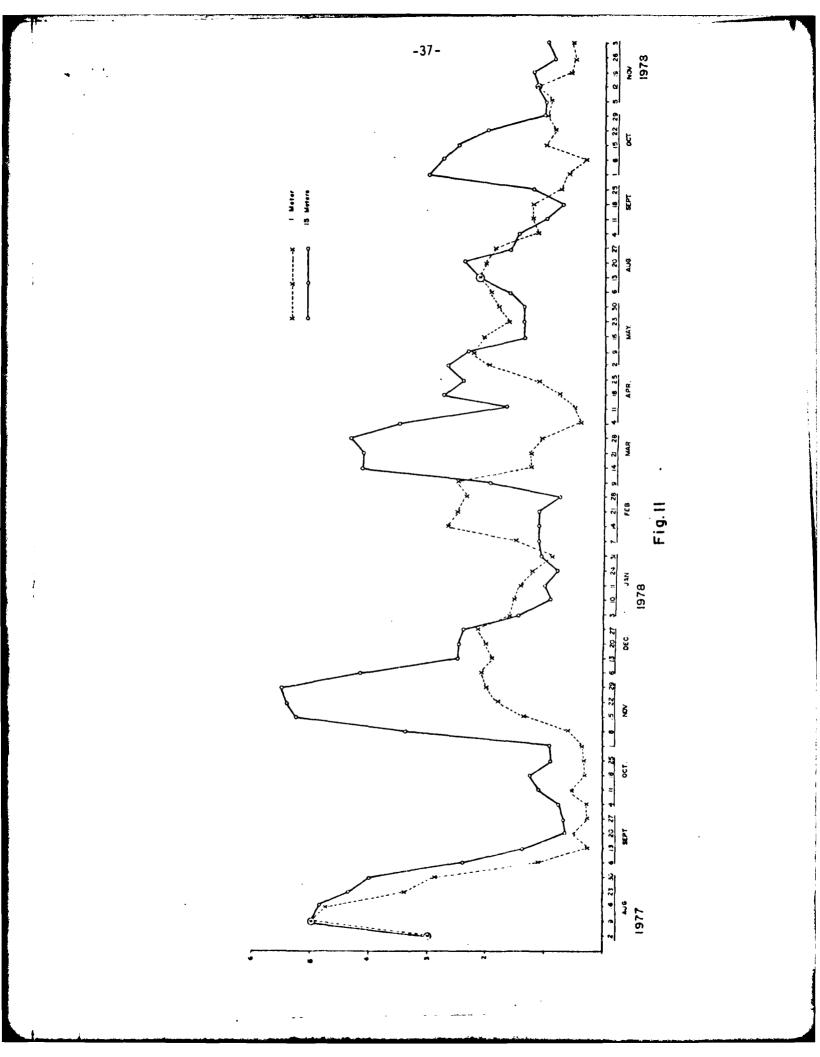
Figures

- Fig. 1-9. Lutzomyia ruii Arias & Young n. sp. 1) Female and male wing.
  2) Spermathecae and genital fork, sperm ducts not visible.
  3) Male genitalia, lateral view. 4) Paramere, a different, larger view than shown in Fig. 3. 5) Genital pump and filaments. 6) Female head. 7) Male flagellomere II showing ascoids.
  8) Male head. 9) Female cibarium. All figures drawn from specimens found at Reserva Ducke. Scale in mm.
  Fig. 10. Spermatheca of Lutzomyia ubiquitalis from Belém, Pará, Brazil.
- The other spermatheca was not drawn. Scale in mm.
- Fig. 11. Seasonal distribution of <u>Lutzomyia ruii</u> n. sp. based on light trap catches at 1 m and 15 m heights above ground at Reserva Ducke (1977-1978).

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A NEW PHLEBOTOMINE SAND FLY IN THE Lutzomyia flaviscutellata COMPLEX FROM NORTHERN BRAZIL (DIPTERA: PSYCHODIDAE)<sup>1</sup>

> D.G. Young<sup>2</sup> J.R. Arias<sup>3</sup>

Sand flies of the Lutzomyia (Nyssomyia) flaviscutellata complex are important as vectors of Leishmania mexicana to small mammals and man in the neotropics. Lainson and Shaw (1968) and Ward et al. (1973, 1977) incriminated Lu. flaviscutellata (Mangabeira) as the vector of Leishmania mexicana amazonensis in Brazil. Tikasingh (1975) recovered this parasite, or a similar one, from wild caught flaviscutellata females in Trinidad. Lu. olmeca clmeca (Vargas and Nájera) in Mexico and Central America and Lu. o. bicolor Fairchild and Theodor in Panama are proven or suspected vectors of Leishmania mexicana mexicana and L. m. aristedesi, respectively. Lainson and Shaw (1979) reviewed the epidemiology of these diseases, noting that "it appears that subspecies of L. mexicana are principally parasites of sylvatic rodents . . . transmission occurs at ground level, where man also becomes infected."

Aitken et al. (1975) isolated Pacui virus and several other arboviruses from wild caught *Lu. flaviscutellata* females near Belém, Brazil. The Disney trap, an animal-baited oil trap routinely used by them and other workers, is excellent for capturing sand flies in this complex (Disney, 1966).

Fairchild and Theodor (1971) and Lewis (1975) provided information on the taxonomy and distribution of these forest sand flies, the latter author observing among other things, "the presence of many erect hairs

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on abdominal tergite 2, while there are none on segment 5. These features and those already known make this complex a remarkably distinct one." The striking coloration of these sand flies, i.e. the pale pleura, coxae and posterior mesonotum contrasting with very dark head, abdomen, and legs, often allows workers to identify specimens without the aid of magnification.

The form described here as a subspecies of *Lu. olmeca* occurs with *Lu. flaviscutellata* at Manaus, Brazil, and vicinity. All measurements are given in millimeters.

Lutzomyia olmeca nociva Young and Arias n.ssp.

Fig. 1-10

<u>Male holotype</u>: Wing length 1.89; width 0.55. Except for pale coxae, pleura, posterior part of mesonotum, rest of insect dark. Head height from vertex to tip of clypeus, 0.36; width 0.33. Eyes large, separated by only 0.04 or by distance = to 2 facet diameters. Interocular suture absent. Flagellomere I, 0.31 long, combined length of II + III = 0.29; ascoids simple, tips of those on II ending before end of flagellomere, on all flagellomeres except last 3. Labrum 0.16 long. Length of palpal segments: 1 (0.027), 2 (0.081), 3 (0.119), 4 (0.054), 5 (0.086); palpal sensilla apparently restricted to middle third of palp 3. Cibarium with about 20 dot-like remnants of teeth, mostly at sides when viewed as in Fig. 10, no cibarial arch or pigment patch. Pharynx 0.19 long, unarmed. Pleura with 7-8 upper and 2 lower episternal setae. Length of wing vein sections: alpha (0.49), beta (0.24), delta (0.10), gamma (0.11). Length of femora, tibiae and basitarsi:

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foreleg, 0.78, 1.30, 0.76; midleg, 0.71, 1.40, 0.93; hindleg, 0.83, 1.50, 1.02. Abdominal tergite 2 with ca. 20 erect hair sockets, tergite 3 with fewer, none visible on other tergites. <u>Genitalia</u>. Style 0.15 long, with 4 major spines, basal pair inserted slightly beyond middle of segment; no subterminal seta. Coxite 0.29 long, without persistent hairs. Paramere simple. Aedeagus subtriangular, well pigmented throughout, acute tip. Genital pump 0.116 long, each filament 0.35 or ca. 3X length of pump. Lateral lobe 0.24 long. Cereus as shown.

Female allotype. Wing length 1.98; width 0.56. Coloration as for  $\sigma$ . Head height 0.47 (including 0.17 long clypeus); width 0.33. Eyes large, separated by 0.05 or by distance = to ca. 2.5 facet diameters. Interocular suture reduced to short stub. Flagellomere I 0.28 long, combined length of II + III = 0.24; ascoids as in  $\sigma$  but absent from terminal 2 flagellomeres. Labrum 0.28 long. Ventral maxillary teeth 25; lateral teeth 13. Length of palpal segments: 1 (0.035), 2 (0.116), 3 (0.159), 4 (0.060), 5 (0.110); palpalsensilla at middle third of segment. Cibarium with 9 horizontal pointed teeth, a few lateral teeth and ca. 36 ventral teeth, median ones larger than others; cibarial arch complete; pigment patch well infuscated, shaped as shown. Pharynx 0.21 long, unarmed. Pleura with 4 upper and 1 lower episternal setae. Length of wing vein sections: alpha (0.52), beta (0.26), delta (0.06),  $a_{roma}$  (0.09). Length of femora, tibiae and basitarsi: foreleg, 0.83, 1.24, 0.80; midleg, 0.73, 1.47, 0.90; hindleg, 0.83, 1.50, 0.98. Abdominal tergite 2 with 30+ erect hair sockets, tergite 3 with 28, few or no erect setae on other tergites. Spermathecae

-40-

with 9-12 segments increasing in size from base to apex, terminal knob oblong, its length at least twice width; individual ducts nearly as long as common duct. Stem of genital fork very broad, blade-like.

Type Data (All material from Amazonas State, Brazil). Holotype σ.
26 km E of Manaus at Reserva Ducke, 19 March 1979, flight trap,
D.G. Young. <u>Allotype</u> ?. 243 km E of Manaus at Rio Urubu, 13
March 1979, flight trap, D.G. Young, J.R. Arias et al. <u>Paratypes</u>.
29?, 30 km E of Manaus, 3 Oct. 1974, rat-baited Disney trap, J.R.
Arias. 3σσ, Mauá, Estrada do Aleixo (km 10), Manaus, 18 Sept.
1974, lizard-baited Disney trap, J.R. Arias. 3σσ, 189?, 243 km
E of Manaus at Rio Urubu, 12-14 March 1979, light and flight traps,
human bait, D.G. Young, J.R. Arias et al. 8σσ, 129?, 26 km E of
Manaus at Reserva Ducke, 16-19 March 1979, flight and light traps,
D.G. Young, J. Vidal and R. Alves de Freitas. 2σσ, 39?, 43 km NE
of Manaus at Reserva Campinas, 23 March 1979, light and flight
traps, D.G. Young, J. Vidal and R. Alves de Freitas. The subspecific name, "nociva," meaning hurtful or injurious, refers to
the bloodsucking habit of the females.

## Discussion:

The flaviscutellata complex of the subgenus Nyssomyia Barretto now consists of 5 named and 1, or possibly 2, unnamed forms. These are: Lu. flaviscutellata (Mangabeira, 1942) from South America, Lu. olmeca clineca (Vargas and Diaz-Najera, 1959) from Mexico and Central America, Lu. o. bicolor Fairchild and Theodor, 1971, from Costa Rica to northern South America, Lu. o. nociva n.ssp. from North Central Brazil, and Lu. inormata Martins, Falcão and da Silva, 1965, from Brazil. Specimens

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from Salvador, Bahia, Brazil (Sherlock and Carneiro, 1962, not *flaviscutellata* Mang.) and from Itiatuba, Pará, Brazil (Lewis, 1975) require further study before their status can be determined.

Lu. inornata, known from only the male, is structurally similar to *flaviscutellata* but the scutellum is dark rather than pale according to Martins et al. (1965). This feature, if consistent, also serves to separate this species from the subspecies of *olmeca*. Through the kindness of M. Emile Abonnenc and Prof. Nicole Leger, we examined a male, tentatively identified and recorded as *inormata* from Oyapock, French Guiana (Leger et al., 1977, Fig. 2). We believe, however, that the specimen is conspecific with *flaviscutellata* because the scutellum is paler than the mesonotum, palp 5 is shorter than palp 3, and the rounded tips of the genital filaments are similar to those of some *flaviscutellata* males from Pará, Brazil. These males, unlike those of *olmeca* and subspecies, have large genital pumps which are almost as long as the lateral lobes (Table 1).

Palpal segment 5 of Lu. o. bicolor and Lu. o. nociva males is subequal in length to palp 2; whereas palp 5 of Lu. o. olmeca is nearly as long as palp 3. The male of nociva is smaller than the other subspecies based on the length of the wings, flagellomeres, and lateral lobes of the genitalia. Also the narrow interocular distance of nociva males is useful in separating this subspecies from the others (Table 1).

Features of the spermathecae, cibarial armatures, palpi, and antennae are diagnostic for the *flavisoutellata* complex females (Fairchild and Theodor, 1971). The broad terminal knobs of the spermathecae of *Lu. o. olmeca* and the *flavisoutellata* complex female of Sherlock and Carneiro (1962) differ from the oblong knobs of the other females,

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especially *nociva* (Figs. 10, 11, 12). Females of *nociva* and the nominate subspecies are distinguished from the others in the complex in having the individual and common sperm ducts subequal in length. The individual sperm ducts of the other females are markedly shorter than the common duct. Additional measurements and meristic characters for each taxon are shown in Table 1.

It should be noted that the broad, blade-like stem of the genital fork reaches its greatest development in *nociva* females. Not all are as wide as that shown in Fig. 7, but this is apparently due to viewing the structure at an improper angle.

The status of Lu. o. nociva may change after more is learned about its geographic distribution, especially in relation to that of Lu. o. bicolor which occurs in the Amazon basin at Leticia, Colombia, and Napo Province, Ecuador (Young, 1979). At present, there is no evidence indicating that the olmeca subspecies have sympatric distributions. Lu. flaviscutellata, however, does occur with two of the olmeca subspecies in Amazonia, supporting its position as a valid species and raising interesting questions about resource partitioning and disease relationships.

#### Acknowledgments

Mr. Alves de Freitas and Mr. João Ferreira Vidal collected and prepared many of the specimens. We also thank Prof. N. Leger and M. Emile Abonnenc for sending us phlebotomines from French Guiana for study.

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Taxon	Lu. flavincutellata	ellata	Lu. inomata	Lu. olme	Lu. olmeca olmeca	Lu. olmeca bicolor	a bicolor	Lu. olme	Lu. olmeca nociva
haracter	J.C	ð ð	ď	ילכ	\$\$	ର୍ଟ୍	\$\$	ଟ୍ଟ	0+ 0+
itstance	$\begin{array}{llllllllllllllllllllllllllllllllllll$	0.050- 0.068 (x = 0.62) n = 10	د.	$\begin{array}{l} 0.098 \\ 0.110 \\ \overline{(x)} = 0.105 \end{array}$ $n = 10$	$\begin{array}{llllllllllllllllllllllllllllllllllll$	0.088- 0.096 (x = 0.090) n = 10	$\begin{array}{llllllllllllllllllllllllllllllllllll$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{l} 0.050 - \\ 0.060 \\ (\overline{x} = 0.053) \\ n = 10 \end{array}$
ength of lagello- ere II	0.29-0.32 0.30-0.33	0-0.33	0.28	0.53	0.37-0.42	0.48	0.31-0.38	0.31 n = 8	0.27-0.32 n = 13
umber of orizontal eeth in ibarium	6-7		:	}	10-12		8-9 (rarely 7 or 10)	1	9-12 9-12 10) 13 13 13
ength of enital ump	0.20		0.19	0.13-0.16		0.14	:	0.11 n = 8	
ength of ateral obe	0.20		0.20	0.40	ł	0.32	1	0.22-0.23 n = 8	:

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Measurements and counts of some features of the *fLaviscutellata* complex species (from Fairchild and Theodor, 1971; Martins et al., 1965 for *Lu. inornata*; this paper where sample size (n) is given). Measurements in millimeters. ble 1.

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## Footnotes

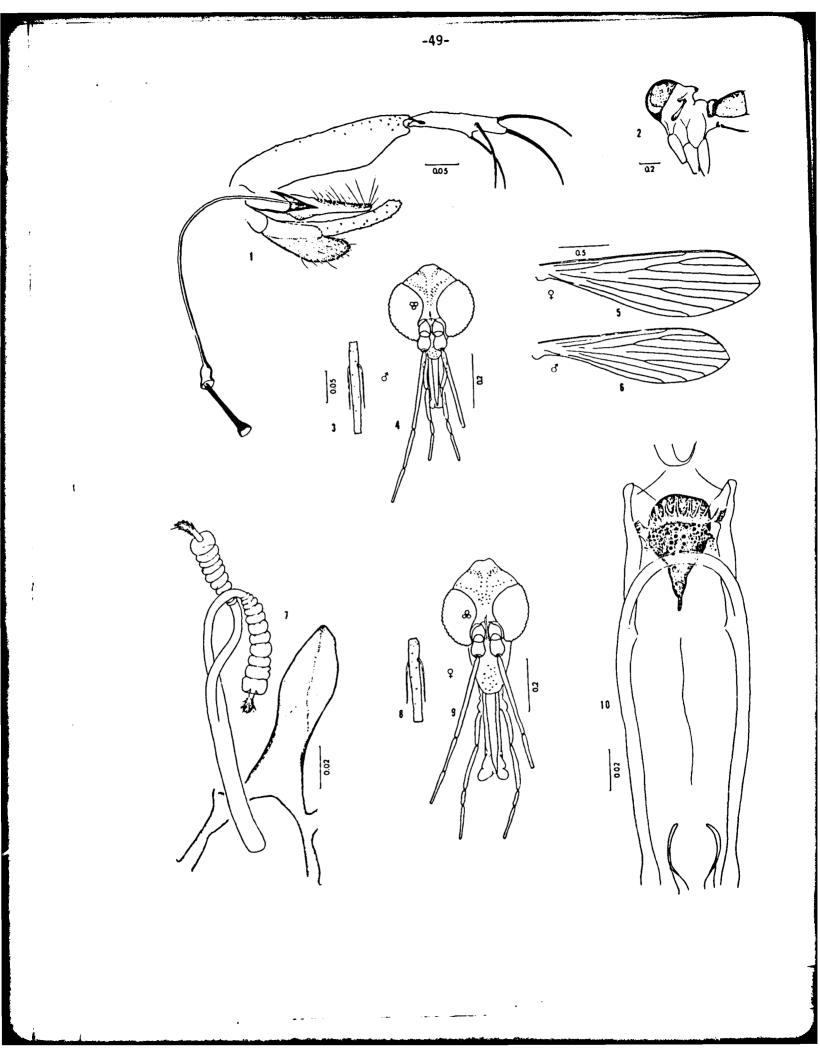
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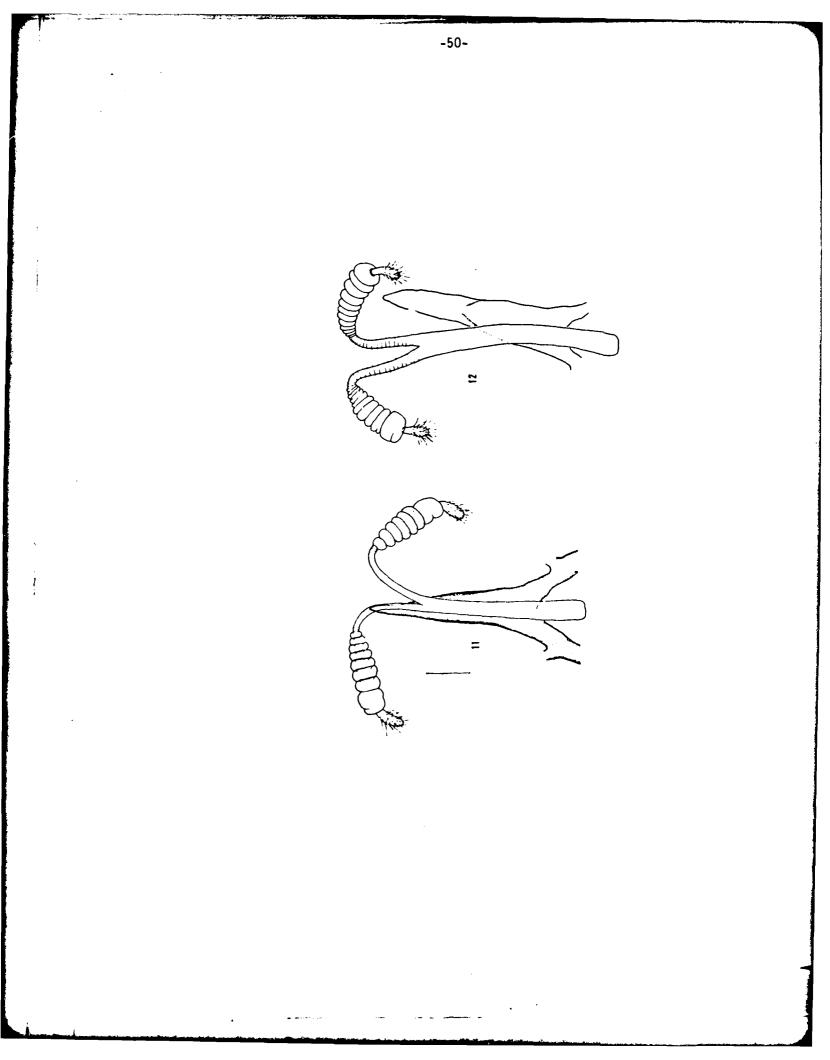
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## Figures

- Figs. 1-10. Lutzomyia olmeca nociva n.ssp. 1) Holotype genitalia, lateral view. 2) Thorax and anterior abdomen of female.
  3) Male flagellomere II. 4) Male head. 5) Female wing.
  6) Male wing. 7) Spermathecae and genital fork. 8) Female flagellomere II. 9) Female head. 10) Female cibarium. Scale in mm.
- Fig. 11. Spermathecae of Lutzomyia flaviscutellata of female collected with Lu. olmeca nociva at Rio Urubu, Brazil.
- Fig. 12. Spermathecae of *Lutzomyia olmeca bicolor* (allotype). Figures 11 and 12 drawn at same scale as Fig. 7.

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### Appendix IV

## A Larval Diet for Rearing Phlebotomine Sand Flies (Diptera: Psychodidae)

Among the major problems in rearing phlebotomine sand flies is excessive larval mortality caused by fungal growth, improper diet or moisture, disease and/or other factors (Killick-Kendrick, R. 1978. Acta Tropica 35:297-313). None of the 600 or so species have been mass reared and only 6 or 7 have been reared in large numbers for more than 10 consecutive generations (Killick-Kendrick, op. cit.).

We have found that an aged 1:1 mixture (by wt. or vol.) of dry rabbit feces and Purina Rabbit Chow complete Diet #6315 (or PuriNa Horse Chow Checkers #350) is an excellent diet for the larvae. The feces were obtained from laboratory rabbits fed on the Lab Rabbit Chow. Our attempts to rear four <u>Lutzomyia</u> spp. from the southern U.S.A. have been successful. We currently maintain closed colonies of <u>Lutzomyia</u> sp. near <u>cruciata</u> (Coq.), an autogenous strain (12 generations), <u>L. anthophora</u> (Addis) (6 generations) and <u>L. shannoni</u> (Dyar) (4 generations). We reared <u>L. vexator</u> (Coq.) in 1976 but purposely abandoned the colony after 4 generations.

The larval food is prepared by grinding the feces and whole chow into small pieces with a mortar and pestle. The final size of the particles ranges from 0.01 to 2.0 mm in diameter, the majority being about 0.5 to 1.0 mm. The dry mixture is evenly spread over the bottom of a petri dish or similar container to a depth of 0.5 to 1.0 cm, saturated

<sup>&</sup>lt;sup>1</sup>The chemical composition of these diets is available from the Ralston Purina Co., Checkerboard Square, St. Louis, Missouri 63188. Changes in composition during and after ageing due to microorganisms are not presently known.

with distilled water and placed in a glass desiccator at 22-30°C with 100% R.H. It is not sterilized. A crude inoculum of spores of the Common bread mold, <u>Rhizopus</u> sp., is transferred from older diet to fresh using a wooden applicator. The desiccator is then covered with a tight fitting lid to prevent entry of phorid flies and other arthropods. The mixture is allowed to age for a month or more (Safyanova, V.M. 1964. Bull. Wld. Hlth. Org. 31:573-576). It is ready for use after most or all of the visible fungi disappears and when the odor becomes similar to that of rich humus. This ageing process eliminates the problem of excessive fungi which, by sheer density, can immobilize and kill larvae.

We rear 1 to 70 larvae in 7 dr (25 ml) polystrene snap-cap vials at temperatures ranging from 23-27°C and 80-100% R.H. The bottom half of each vial is filled with plaster of paris, allowed to dry, then saturated with distilled water. Gravid sand flies, usually 1 per vial, oviposit in these containers. The moist, aged medium is introduced into the vials anytime before egg hatch (about 1 g of medium for 50 eggs). As the larvae grow, we do not remove their feces but sometimes add more food or water. Little else is required for their care. Larval mortality, consistently less than 10%, results from factors other than improper nutrition such as accidents due to handling or excess water.

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