

# Trypanosomes and Other Microorganisms from Panamanian *Phlebotomus* Sandflies

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# Trypanosomes and Other Microorganisms from Panamanian *Phlebotomus* Sandflies\*

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**ABSTRACT:** One Panamanian species of sandfly, *Phlebotomus vespertilionis*, usually associated with bats, has a high overall rate of infection (67.9%) with a trypanosome. Both crithidial and trypanosomal forms are found in the mid- and hindgut but not in the foregut or mouthparts. In the hindgut there may be large numbers of the dominant crithidial forms attached to the epithelium. It has not been determined whether or not the sandfly trypanosome is the same as that occurring in associated bats. Trypanosomes were also found in another species, *P. trinidadensis*, but at a much lower rate (3.3%). Occasional infections of the hemocoel with gregarines, fungi, and nematodes have been noted in a number of species.

During an extensive series of dissections of wild-caught Panamanian *Phlebotomus* with the primary objective of determining the extent and kind of leptomonad flagellate infections in guts of wild sandflies, a variety of other organisms was encountered. The leptomonad flagellate infections have been discussed by Johnson, McConnell, and Hertig (1963). The purpose of the present paper is to give our observations on the other organisms found in and on wild sandflies of our series.

Other investigators have found a number of organisms aside from leptomonad flagellates associated with *Phlebotomus*, including mites, fungi, tapeworm larvae, nematodes, gregarines, and trypanosomal and crithidial flagellates (among others, Adler and Mayrink, 1961; Herrer, 1942; Lewis and Minter, 1960; Shortt and Swaminath, 1927; Subramaniam and Naidu, 1944). In our series we found all except tapeworm larvae. Of special interest are the trypanosome infections and the bulk of our discussion will deal with these.

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## Trypanosomes

Little is known of the role played by various species of *Phlebotomus* in the transmission of trypanosomes to their vertebrate hosts. Lewis and Minter (1960) mentioned the presence of trypanosomes in the crithidial stage in the rectum of females of African *Phlebotomus* (*Sergentomyia*) *garnhami* Heisch et al. Whether the flagellates observed in *garnhami* were obtained from the blood of a vertebrate host and are thus members of the genus *Trypanosoma* is unknown.

Adler and Theodor (1935) demonstrated that sandflies can transmit *Trypanosoma platydaetyli* to the gecko *Tarantola mauritanica*, probably by being ingested by the lizard, although bite transmission was not ruled out. Infections with *T. platydaetyli* developed in 40 of 43 *Phlebotomus parroti* Adler and Theodor fed on an infected gecko. The flagellates adopted an anterior position in the cardia and had progressed forward into the esophagus within 3 or 4 days, but in the 9-day observation period none were seen in the mouthparts. Flagellates were never seen in the hindgut.

In Peru two or more species of *Phyllotis*, a cricetid rodent, are often found infected with *Trypanosoma phyllotis* Herrer. Herrer (1942) found that females of *Phlebotomus naguchii* Shannon, which often inhabit the burrows of these mice, had an extremely high flagellate infection rate. As many as 80% of wild-caught

females taken from burrows of infected *Phyllotis* and still containing parts of a blood meal were infected, and this species could be readily infected in the laboratory by feeding on infected *Phyllotis*. In both naturally and experimentally infected sandflies the trypanosome was always in the crithidial stage, and by the 4th or 5th day extremely heavy infections were found in both the stomach and cardia. Flagellates at times extended into the pharynx and buccal cavity, but never were seen in the mouthparts. Crithidia were expelled with the feces, and by the time all evidence of the blood meal had disappeared no flagellates remained in the gut.

In Panama we have found females of two *Phlebotomus* species infected with flagellates of the genus *Trypanosoma*. Both crithidial and trypanosomal stages were found in the gut of infected insects. In a large number of infections flagellates were present in the blood meal. No infected males were found. In one instance 43 of 44 female *P. vespertilionis* Fairchild and Hertig were infected but none of 45 males of this species taken at the same time and place harbored any type of flagellate.

Seven of 215 (3.3%) female *trinidadensis* Newst. dissected were infected with trypanosomes. Possibly some of the infections were acquired from lizards which we know serve as one source of blood meals for *trinidadensis*. However, in one of the seven infected females flagellates were found in a fresh blood meal from a mammal, i.e., the red cells were non-nucleated.

Most of our trypanosome infections were in *P. vespertilionis*. A closely related species, *isovespertilionis* F. and H., cannot be separated in the female, and it is possible this species is also infected. However, males of *isovespertilionis* were taken in association with infected females very rarely and only in one locality, and for the sake of convenience we shall refer to all infected females as *vespertilionis*.

Our series of collections of *vespertilionis* came from hollow logs and trees on the Pacific side of the Isthmus, and from tree buttresses, hollow trees, and rock caves in the Continental Divide area. Everywhere *vespertilionis* was found, infected individuals were taken. The overall infection rate was high, with 67.9%,

or 747 of 1,101 females dissected, infected with trypanosomes.

Although we have no definite information on feeding preferences of *vespertilionis*, this species is always found in places inhabited by bats. Since all of 24 females whose fresh blood meals were smeared and stained contained only nonnucleated erythrocytes, the sources of its blood meals must be mainly mammalian. Fifty of 144 (29%) female *vespertilionis* collected at various times from a hollow tree inhabited by several genera of bats were found infected with trypanosomes. We captured eight bats of the genus *Carollia* from the same tree and bled them by cardiac puncture. Five of the eight bats were positive for trypanosomes by culture of the heart blood.

Over a period of a year weekly collections of resting *Phlebotomus* were made in one or more of several small rock caves near Quebrada Bonita in the Continental Divide area. The majority of female sandflies taken were *vespertilionis*. Bats were always present in each cave and *Carollia perspicillata* was the most common bat species. *C. perspicillata* was the only mammal taken from cave no. 6 which had the highest percentage of infected female *vespertilionis* (82%). In cave no. 6 all but one of 320 female sandflies captured were *vespertilionis*. The remaining female was a *trinidadensis* infected with trypanosomes.

Within the sandfly the flagellates are found in the mid- and hindgut but never in the foregut or mouthparts. A fly with an infected blood meal may also have a heavy infection in the hindgut, probably the result of a previous blood meal. Flagellates have never been observed in the midgut in the liquid phase of the blood meal which may appear outside the peritrophic membrane, and we assume that the membrane, while still intact, also prevents passage of the flagellates to the hindgut.

Very large round leptomonad forms are occasionally seen in the blood meal during the first 2 days following the infective feed, but large crithidial forms averaging 21.9  $\mu$  in length are always the dominant forms. Smaller trypanosomal forms with an average length of 11.4  $\mu$  are present but relatively rare. Free motile trypanosomal and crithidial forms are common in the midgut after all signs of the

blood meal disappear. Rarely, trypanosomal and crithidial forms may be found in the Malpighian tubules.

In the hindgut crithidia are dominant also. The hind triangle (the part of the hindgut just posterior to the midgut which is triangular in optical section) often has only round, non-motile crithidia lining the walls. The flagellum of this type is always short, and in Giemsa-stained smears the free portion of the flagellum may appear merely as a blob of pink-staining flagellar material at the anterior end of the body plus a well-formed undulating membrane and accompanying flagellum joining the blob to the kinetoplast. At varying distances below the hind triangle a thick or thin belt of longer and thinner but also almost aflagellar crithidia averaging  $10.8 \mu$  in length may be palisaded on the walls so thickly that they occlude the lumen (Fig. 3). In the rectal ampulla similar relatively thin crithidia usually cover the rectal glands (Figs. 1, 2). Infected flies may lack attached flagellates in any one or two of the three typical places of attachment, i.e., the hind triangle, hindgut proper, or the rectal glands. In infections we assume may be advanced, crithidia in the hind triangle are motile and oval or rather thin. Also in advanced infections the lumen of the entire hindgut, though especially in the less occluded hind triangle and rectal ampulla, may be filled with extremely motile, very small, usually C-shaped to round, trypanosomal forms averaging  $7.2 \mu$  in diameter or length. Clouds of these small trypanosomes are often released into the saline dissecting solution by contractions of the hindgut after or during the process of withdrawing the gut from the abdomen of the fly.

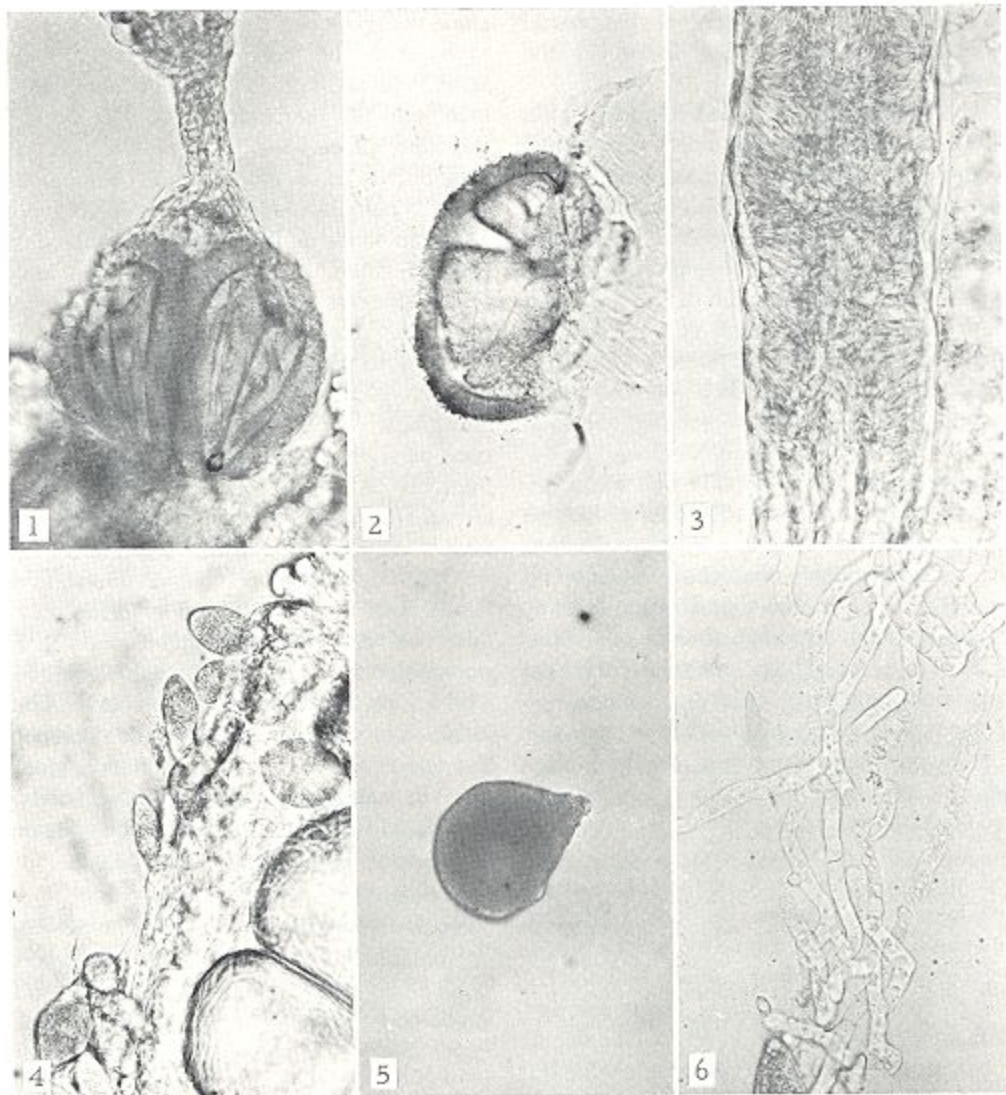
Identity of the flagellates found in *P. vespertilionis* remains to be established. The trypanosomes of both the sandflies and the five infected *Carollia* are probably not *Trypanosoma vespertilionis* (Battaglia). Zeledón and Vieto (1957) experienced no difficulty in culturing and subculturing *T. vespertilionis* recovered from the heart blood of infected *Glossophaga soricina*. These authors also found trypanosomes in a blood smear from one of five *Carollia perspicillata*. Dr. Zeledón has informed us that they did not attempt to culture the *Carollia* trypanosome. They as-

sumed it to be the same as the one from *Glossophaga* on the basis of morphology, negative xenodiagnosis, failure to infect laboratory animals, and the fact that all the bats came from the same place. We were unable to establish subcultures from any of our *Carollia* strains using Senekjic's modification of NNN medium, although some multiplication took place in the original tubes. Also, we have had severely limited success in culturing the flagellates from over 100 infected *P. vespertilionis* guts. In addition to Senekjic's medium we used neoptone-blood-agar as formulated by Wallace and Clark (1959) plus differing overlay solutions of Locke's rich in sucrose or trehalose. Survival of trypanosomes in culture was obtained several times but multiplication did not take place and subcultures were unsuccessful.

The morphology of *T. vespertilionis* and the Panamanian flagellates from sandflies may also differ. *T. vespertilionis* trypanosomes in blood smears average  $16.0 \mu$  in length, ours average  $11.4 \mu$ . Trypanosomes in Giemsa-stained smears of blood meals from *Phlebotomus vespertilionis* had the nucleus slightly posterior to the middle. In contrast, *Trypanosoma vespertilionis* in a blood film from infected *Glossophaga* had the nucleus slightly anterior to the middle (Zeledón and Vieto, 1958).

Experiments to determine the relationship of the sandfly *Trypanosoma* to that of *Carollia* must await the solution of our difficulties in culturing the flagellates and in persuading laboratory-reared *P. vespertilionis* to take a blood meal from any source. To date, seven unsuccessful attempts have been made to feed laboratory-reared *vespertilionis* on bats (*Carollia perspicillata*, *Cormura brevirostris*, and *Pteropteryx kappleri*). Three attempts to feed this sandfly on a guinea pig, an opossum (*Philander*), and a spiny rat (*Proechimys*) were also unsuccessful.

*Phlebotomus vespertilionis*, if involved in transmitting trypanosomes to a vertebrate host, does not do so by bite since anterior infections are not found. The presence of small trypanosomes in the hindgut which may be expelled from the gut in droplets of liquid leads us to speculate that these trypanosomes may be metacyclic forms which could cause infection in a vertebrate host by contaminating superficial skin lesions or the bite wound.



FIGURES 1-6. Micromorganisms from Panamanian sandflies. 1. Rectal glands covered with crithidiform flagellates. The glands are in situ within the rectal ampulla of the hindgut. *P. vespertilionis*, female.  $\times 240$ . 2. Rectal glands as above, dissected out of the ampulla. Note the loose flagellates to the left in the dissecting medium. *P. vespertilionis*, female.  $\times 240$ . 3. Palisaded crithidiform flagellates lining the hindgut. *P. vespertilionis*, female.  $\times 400$ . 4. Protozoa, presumably gregarines, attached to the exterior surface of the midgut. This individual also had free adult gregarines in the hemocoel. *P. trapidoi*, female.  $\times 240$ . 5. Adult gregarine from the hemocoel. The pale nucleus is visible near the center of the organism. The conical extension on the right side is capable of limited expansion and contraction. *P. camposi*, male.  $\times 240$ . 6. Hyphae of a fungus found in the hemocoel. *P. sanguinarius*, female.  $\times 240$ .

#### Gregarines

Acephaline gregarines of one or more species were found in the hemocoel of ten species of sandflies: *camposi* Rodr. 2♂, *cruciatus* Coq. 1♀, *gomezi* Nitz. 3♂ 3♀, *hartmanni* F. and H. 2♀, *panamensis* Shannon 1♀, *sanguinarius* F.

and H. 4♀, *shannoni* Dyar 1♀, *trapidoi* F. and H. 2♀, *trinidadensis* 1♀, *ylephiletor* F. and H. 3♀. It is apparent that gregarines are not common parasites of Panamanian sandflies since only 18 of more than 6,000 females dissected were found infected. The gregarines

TABLE I. *Fungus infections in Phlebotomus species.*

Sandfly species	Resting females			Biting females		
	No. diss.	No. pos.	Per cent pos.	No. diss.	No. pos.	Per cent pos.
<i>camposi</i>	17	2	11.8	—	—	—
<i>gomezi</i>	27	1	3.7	636	0	0
<i>ovallesi</i>	7	5	43.0	7	0	0
<i>panamensis</i>	24	0	0	622	4	0.5
<i>sanguinarius</i>	8	0	0	1,623	6	0.2
<i>shannoni</i>	60	6	10.0	34	0	0
<i>trinidadensis</i>	215	8	3.7	—	—	—
<i>vespertilionis</i>	1,101	224	20.0	—	—	—
<i>vexillarius</i>	10	1	10.0	4	0	0
<i>ylephiletor</i>	439	18	4.2	359	0	0
Total	1,908	263	13.8	3,285	10	0.3

are apparently well tolerated by sandflies. Fifteen of the 18 infected females were captured when attempting to take a blood meal, an indication of normal physiology. Laboratory-reared flies have never been found infected.

Usually infections consisted of one to ten single adults, although occasional infections were heavier and included mature gametocysts. Adult individuals in some infections had a conical projection on one side (Fig. 5). A similar projection was noted by Adler and Mayrnik (1961) in some adults of *Monocystis chagasi*. These authors postulated it might be an organelle of attachment. *Monocystis chagasi* discharges its oocysts into the lumen of the accessory glands of the ovary of its normal host, *Phlebotomus longipalpis* Lutz and Neiva. Alerted by Adler to the possibility that gametocysts of our gregarine species might be attached to the accessory glands, we made a careful search of the glands of both gregarine-infected and noninfected Panamanian female sandflies during our dissection series. Never were gametocysts or their remaining empty envelopes found attached to the glands nor were oocysts seen within the gland lumen. However, at times what appeared to be gregarines were found attached to the outer surface of the midgut (Fig. 4) and these are probably a stage in the life history of the gregarine. Such attached organisms were sometimes but not always found in association with free adult gregarines in the hemocoel. The life of the Panamanian gregarines may be similar to that of *Monocystis mackiei*, which probably includes a phase in the gut of its host, *Phlebotomus argentipes* Ann. and Brun. (Shortt and Swaminath, 1927).

### Fungus

The mycelia of fungi occurred in individuals of ten sandfly species: *camposi* ♀♀, *gomezi* ♀♀, *ovallesi* Ortiz ♀♀, *panamensis* ♀♀, *sanguinarius* ♀♀, *shannoni* ♀♀, *trinidadensis* ♀♀, *vespertilionis* ♂♂ and ♀♀, *vexillarius* F. and H. ♀♀, *ylephiletor* ♀♀. The morphology of the hyphae was similar in all infections (Fig. 6). In both sexes the fungus was almost always associated with the gonads and in heavy infections the mycelium extended throughout the hemocoel. Three attempts to culture the fungus (once from *vespertilionis*, twice from *shannoni*) on both Sabouraud's medium and on blood agar were without success.

Table I illustrates the probability that fungus may have a detrimental effect on some host species. In most species the proportion of fungus infections was higher in resting than in biting females. We assume the fungus may disturb the normal physiology in some way so as to prevent the female from seeking a blood meal. The difference was especially marked in *ylephiletor* which had 4.2% of 439 resting females infected with fungus while none of 359 females taken while biting had fungus. In the case of *vespertilionis* 58% of the fungus-infected females also had flagellate infections. This indicated that at least that number had fed and thus had not been adversely affected by the presence of the fungus or its degree of development at the time of feeding. Six of 72 (8.4%) *vespertilionis* males were also infected with fungus.

### Miscellaneous

Infections consisting of a single rather large nematode of undetermined family were found

three times in the hemocoels of female *sanguinarius*, *vespertilionis*, and *panamensis*. Larval nematodes and eggs of a species probably related to the genus *Tylenchinema*, family Allantonematidae, were found in the hemocoel of both male and female *vespertilionis* (1 of 72 males and 2 of 1,101 females). The infections were extremely heavy and the abdomens of the infected flies were distended with worms. Nine light infections of 1 to 20 larval nematodes also occurred in *vespertilionis* females. Eight were in females which had had a blood meal, judging from concurrent infections with flagellates. These nematodes may be the same as the *Tylenchinema*-type worms or possibly are larval filariids obtained from the host blood meal.

Mites have been found attached to the exoskeleton of three species of sandflies. Individuals of the family Stigmaeidae were attached by their mouthparts on the thorax and abdomen of several *gomezi* and *P. pius* F. and H. These mites are of undescribed species of the genus *Ledermuelleria*. A deutonymph of the family Uropodidae was taken from a *P. aclydiferus* F. and H. It was attached to the thorax by the anal peduncle.

We are indebted to Dr. F. M. Summers, University of California at Davis, for the identification of the mites.

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