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NEOTROPICAL SAND FLIES (DIPTERA: PSYCHODIDAE), INVERTEBRATE HOSTS OF *ENDOTRYPANUM SCHAUDINNI* (KINETOPLASTIDA: TRYPANOSOMATIDAE)[1]

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Abstract: Trypanosomatid flagellates were recovered from 58 (81%) of 72 Panamanian sloths by using the biopsy-culture technique; 21 (29%) of the animals were infected with *Endotrypanum schaudinni* to the exclusion of other trypanosomatids. Three species of laboratory-reared sand flies, *Lutzomyia sanguinaria*, *L. gomezi* and *L. trapidoi*, acquired *E. schaudinni* from the sloths during xenodiagnostic studies. Infections in the sand flies were verified in dissections from 1 to 23 days following acquisition feedings. The flagellates, all of which appeared to be promastigotes, were distributed from the cardia to the rectal ampula. Highest concentrations of flagellates were found consistently in the pylorus. Ovoid motile and nonmotile forms, attached to the gut wall, dominated the infections. Lesser numbers of elongated free-swimming forms were present in the gut lumen of most infected flies. Laboratory and field data indicate that several species of *Lutzomyia* are involved in the transmission cycle of this parasite.

Endotrypanum schaudinni Mesnil & Brimont, 1908 is an intraerythrocytic parasite (FIG. 1) of sloths. A variety of hematophagous arthropods associated with sloths have been studied as potential vectors, without success. Flagellate infections could not be detected from any of the following arthropods: *Rhodnius prolixus* fed on culture forms mixed with guinea pig blood (Cunha & Muniz 1944); *Amblyomma gertschi* collected from infected *Bradypus griseus griseus*, and *Triatoma dimidiata* and *T. infestans* fed on an infected *Choloepus didactylus* (Montero-Gei 1956); *Amblyomma geayi*, *Panstrongylus megistus*, *P. geniculatus*, *Triatoma brasiliensis*, *T. sordida*, *Clerada apicicornis*, *Anopheles argyritarsis*, *Psorophora cingulata* and *Phoniomyia pilicauda* fed on infected *C. didactylus* (Deane 1961); *R. prolixus*, *Triatoma gerstaeckeri*, *T. infestans* and *T. phyllosoma pallidipennis* fed on an infected *C. didactylus* (Packchanian & Najarian 1962); and mites of the genus *Edentalges* collected from *Choloepus hoffmanni*, and *T. dimidiata* and *Haemagogus lucifer* fed on infected *C. hoffmanni* (Shaw 1969).

Deane (1961) reported survival of flagellates in *Culex* mosquitoes (species not determined) and *Aedes scapularis* at 48 hr after feeding on infected *C. didactylus*, and in the tabanid *Dichelacera januarii* at 84 hr after feeding on the same host. Shaw (1964, 1969), working in collaboration with scientists at Gorgas Memorial Laboratory in Panama, pioneered studies which first incriminated phlebotomine sand flies as potential vectors of *E. schaudinni*. Hematophagous insects were collected from man and from *C. hoffmanni* (later found infected with *E. schaudinni*) on a 30-ft (9-m) platform in Achiote, Panama; dissections of the sand flies obtained showed flagellate

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infection rates of 21.5% and 52.2%, respectively. Shaw concluded that the difference in infection rates undoubtedly was due to the fact that some of the flies collected from the sloths became infected with *E. schaudinni*. It should be noted, however, that we later found 13 (37.1%) of 35 *C. hoffmanni* collected in Achiote infected with *Leishmania braziliensis* (Herrer et al. 1973). Because of the nature of the infections in the flies collected from his sloth bait, Shaw concluded that 6 of the 17 positive flies (2 *Lutzomyia trapidoi*, 3 *L. sanguinaria* and 1 *L. gomezi*) harbored *E. schaudinni*. He also reported 2 of 8 laboratory-reared *L. sanguinaria* positive for this parasite, 1 at 10 hr and the other at 6 days after feeding on an infected sloth. The finding that *E. schaudinni* could survive for 6 days in the sand fly was the first solid evidence incriminating *Lutzomyia* in the natural transmission cycle of this parasite. The involvement of *Lutzomyia* species in the life cycle of other vertebrate trypanosomatids in the New World has been well documented (TABLE 1).

During the past several years we have had the opportunity to feed laboratory-reared sand flies on Panamanian *C. hoffmanni* in xenodiagnostic studies. This report tabulates our findings as they concern *E. schaudinni*.

MATERIALS AND METHODS

The techniques for feeding clean sand flies on freshly captured sloths, and the subsequent processing of these insects during xenodiagnosis trials, were published previously (Christensen & Herrer 1972). The biopsy-culture method (Herrer et al. 1966) was used to detect leishmanial and hemoflagellate infections in the edentates. Fresh blood preparations and blood smears stained with Giemsa were screened for intraerythrocytic forms of *E. schaudinni* and other hemoflagellates. The presence of intraerythrocytic flagellates and the cultivation of promastigote forms which failed to infect Golden Hamsters were criteria used for diagnosing *Endotrypanum* infections in sloths. Animals with multiple hemoflagellate infections were not utilized in the present study.

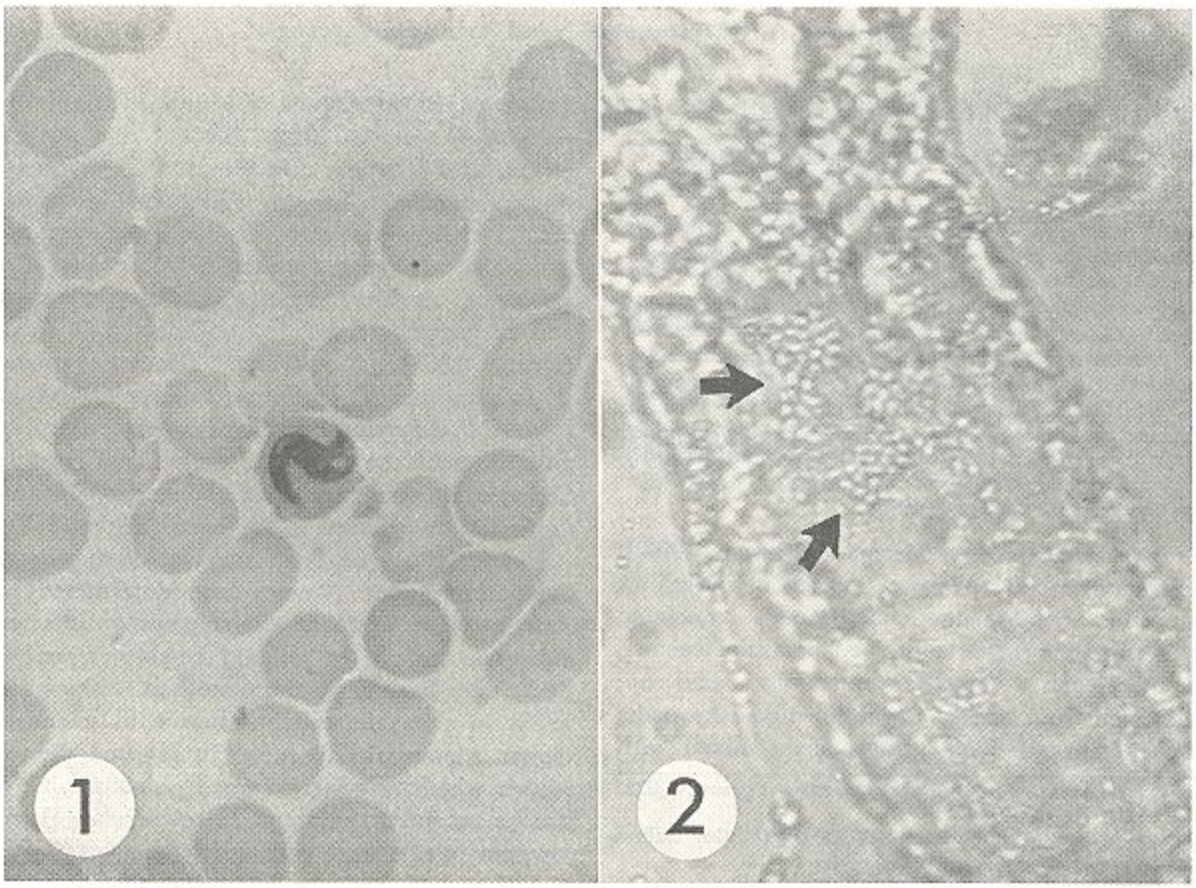

FIG. 1–2. (1) Intraerythrocytic stage of *Endotrypanum schaudinni* from the blood of the Two-toed Sloth, *Choloepus hoffmanni*, stained with Giemsa. (2) Attached ovoid flagellates of *Endotrypanum schaudinni* in the pylorus of *Lutzomyia gomezi* (arrows) 8 days after feeding on an infected sloth.

TABLE 1. The association of New World phlebotomine sand fly species with vertebrate Trypanosomatidae.*

Vertebrate host	Parasite	Sand fly vector and/or intermediate host	Reference
Phyllotis darwinii limatus	*Trypanosoma phyllotis*	*Lutzomyia noguchii*	Herrer (1942)
Saccopteryx bilineata	*T. leonidasdeanei*	*L. vespertilionis*	Zeledón & Rosabal (1969), Christensen & Herrer (1975)
Sceloporus occidentalis occidentalis	*T. scelopori*	*L. vexator occidentis*	Ayala (1970)
Gerrhonotus multicarinatus	*T. gerrhonoti*	*L. vexator occidentis*	Ayala & McKay (1971)
Thecadactylus rapicaudus	*T. thecadactyli*	*L. trinidadensis*	Christensen & Telford (1972)
Bufo boreas halophilus	*T. bufophlebotomi*	*L. vexator occidentis*	Anderson & Ayala (1968)

*Excluding *Leishmania*.

RESULTS

Dissections of 1665 laboratory-reared *L. sanguinaria*, *L. gomezi* and *L. trapidoi* were made at various periods after having fed on 72 *C. hoffmanni*. A total of 58 (81%) of the edentate hosts had trypanosomatid infections, with 21 (29%) showing pure *Endotrypanum* parasitemias. The acquisition of this parasite by the 3 sand fly species in xenodiagnostic trials is shown in TABLE 2. The data in TABLE 3 were tabulated from 136 positive flies which had fed on 14 *Endotrypanum*-infected animals. Dissections in which any aspect of the alimentary tract was not visible were not incorporated in TABLE 3. The majority of *Endotrypanum* comprising the sand fly infections were rounded or oval forms attached to various sections of the gut wall. These forms usually were clustered in rosettes (FIG. 2) or palisades and exhibited little or no motility. Lesser numbers of unattached actively motile forms were characteristic of most infections. Smears made from the gut of infected flies stained with Giemsa were rather unsatisfactory, since the flagella and internal organelles of the parasite stained poorly. However, those forms clearly identifiable from fresh and stained preparations were promastigotes. The pylorus (=hind triangle) consistently had the greatest concentration of parasites. One *L. sanguinaria* dissected 23 days after feeding showed a single unattached and motile flagellate in the lumen of the cardia, and several free and attached forms in the midgut. The pylorus and intestine of this fly were not seen.

TABLE 2. Phlebotomine sand fly xenodiagnostic trials on captured *Choloepus hoffmanni* naturally infected with *Endotrypanum schaudinni*.*

C. hoffmanni work no.	*Lutzomyia* species	Total/positive (%)
1788	*L. sanguinaria*	29/3 (10)
2110	*L. sanguinaria*	24/0 (0)
2930	*L. sanguinaria*	41/26(63)
2941	*L. sanguinaria*	35/28(80)
2943	*L. sanguinaria*	27/8 (30)
2961	*L. sanguinaria*	1/0 (0)
2962	*L. sanguinaria*	46/8 (17)
2979	*L. sanguinaria*	4/0 (0)
3114	*L. sanguinaria*	14/1 (7)
3219	*L. sanguinaria*	15/0 (0)
3301	*L. sanguinaria*	17/1 (6)
1377	*L. sanguinaria*	13/5 (39)
	L. gomezi	28/18(64)
2097	*L. sanguinaria*	40/7 (17)
	L. gomezi	17/11(68)
2098	*L. sanguinaria*	30/4 (13)
	L. gomezi	26/19(73)
2871	*L. sanguinaria*	9/0 (0)
	L. gomezi	1/0 (0)
2928	*L. sanguinaria*	53/9 (17)
	L. gomezi	16/12(75)
2929	*L. sanguinaria*	32/4 (13)
	L. gomezi	10/10(100)
3027	*L. sanguinaria*	2/0 (0)
	L. trapidoi	4/0 (0)
3203	*L. sanguinaria*	11/0 (0)
	L. trapidoi	4/0 (0)
3220	*L. gomezi*	3/1 (33)
	L. trapidoi	18/12(67)
3112	*L. sanguinaria*	22/0 (0)
	L. gomezi	8/0 (0)
	L. trapidoi	1/0 (0)

*Sand flies dissected from 1 to 23 days following acquisition feedings.

Infection rates in companion lots of *L. sanguinaria* and *L. gomezi* fed on 5 sloths are compared in TABLE 4. *E. schaudinni* was the only parasite isolated from these animals by the biopsy-culture technique. However, flagellates cultured from one of the *L. gomezi*, which had fed on sloth No. 2929, were infective to hamsters. The parasite was identified as *Leishmania braziliensis* (Christensen & Herrer 1972).

DISCUSSION AND CONCLUSIONS

Until now the search for potential vectors of *E. schaudinni* has been rather disappointing. Most of the hematophagous arthropods studied appear unable to acquire the infection from the vertebrate host.

The appearance of flagellates in arthropod blood meals several days following engorgement on infected hosts, as in the case of a *Culex* species and *Ae. scapularis* (Deane 1961), cannot be considered

TABLE 3. Location of flagellates in the gut of laboratory-reared sand flies after feeding on *Choloepus hoffmanni* infected with *Endotrypanum schaudinni*.

Days post-feeding	Species of *Lutzomyia*	Positive flies	Number (%)					
			Cardia	Midgut	Pylorus	Malpighian tubules	Hind gut	Rectal ampulla
1	*sanguinaria*	1	0	1	0	0	0	0
	gomezi	1	0	1	0	0	0	0
2	*sanguinaria*	7	0	7	2	0	2	0
3	*sanguinaria*	1	0	1	1	0	1	0
	gomezi	2	0	2	0	0	0	0
4	*sanguinaria*	7	2	6	1	1	1	1
	gomezi	7	1	4	6	3	5	5
5	*sanguinaria*	18	1	7	11	5	12	3
	gomezi	8	2	5	7	1	7	5
6	*sanguinaria*	13	2	6	5	2	9	3
	gomezi	7	2	2	5	1	4	3
7	*sanguinaria*	25	1	8	16	6	16	4
	gomezi	10	3	6	9	2	7	4
8	*sanguinaria*	11	2	6	10	2	11	7
	gomezi	11	3	4	6	0	2	2
9	*sanguinaria*	2	0	2	2	1	2	2
	gomezi	1	0	0	1	1	0	0
10	*gomezi*	1	0	0	0	0	1	0
11	*gomezi*	3	0	2	3	1	2	1
	Total	136	19(14)	70(52)	85(63)	26(19)	82(60)	40(29)

indicative of vector potential. Such findings do not prove that multiplication of the parasite or its survival after digestion has taken place. However, Deane's report of the survival of *Endotrypanum* in the tabanid *D. januarii* for 84 hr should be investigated further.

Our findings support Shaw's hypothesis (1964) that *Lutzomyia* species probably play an important role in the transmission of *Endotrypanum*. The significantly higher parasite acquisition rate in *L. gomezi* as compared to that in *L. sanguinaria* (TABLE 4) probably indicates that the former species is a more efficient vector of *Endotrypanum*. The 67% infection rate in *L. trapidoi* which fed on sloth No. 3220 (TABLE 2) also implicates this species as a potential vector.

Shaw (1964) noted that 4 sand fly species, *L. trapidoi*, *L. sanguinaria*, *L. gomezi* and *L. ylephiletor*, were collected feeding on sloths during his study in Panama. Tesh et al. (1971, 1972) reported that a proportion of blood-engorged wild-caught *L. serrana*, *L. pessoana*, *L. trapidoi*, *L. shannoni* and *L. panamensis* tested by the precipitin method reacted with edentate antiserum. Our laboratory studies have shown that the latter 4 species, as well as *L. gomezi* and *L. sanguinaria*, feed readily on restrained sloths in cages.

L. trapidoi, *L. panamensis*, *L. sanguinaria*, *L. gomezi* and *L. ylephiletor* have been incriminated as vectors of *Leishmania braziliensis* in Panama (Christensen & Herrer 1973). These 5 *Lutzomyia* species have comprised 95.8% of 93,902 sand flies from our animal-baited collections (unpubl. data). Since the sloth *C. hoffmanni* is the principal reservoir host of *L. braziliensis* (Herrer et al. 1973) as well as *E. schaudinni* in Panama, it is likely that the same sand fly species transmit both parasites in this country.

The distribution of *E. schaudinni* in the intestinal tract of laboratory-reared *L. sanguinaria* and *L. gomezi* appears to spread posteriad as the infection progresses. The greatest concentration of flagellates occurred in the pylorus. In none of the infections were parasites seen anterior to the cardia. Johnson

TABLE 4. Infection rates in companion lots of laboratory-reared *L. sanguinaria* and *L. gomezi* after feeding on *Choloepus hoffmanni* naturally infected with *Endotrypanum schaudinni*.

C. hoffmanni work no.	*L. sanguinaria*			*L. gomezi*			Total		
	No. fed	No. pos.	(% pos.)	No. fed	No. pos.	(% pos.)	No. fed	No. pos.	(% pos.)
1377	13	5	(39)	28	18	(64)	41	23	(56)
2097	40	7	(17)	17	11	(68)	57	18	(32)
2098	30	4	(13)	26	19	(73)	56	23	(41)
2928	53	9	(17)	16	12	(75)	69	21	(30)
2929	32	4	(13)	10	10	(100)	42	14	(33)
Total	168	29	(17)	97	70	(72)	265	99	(37)

& Hertig (1970) reported a similar growth pattern for *L. braziliensis* in these sand flies after feeding on infected hamsters. They noted that there is no evidence as to how growth in the hindgut can contribute to the transmission of *L. braziliensis*. Since anterior growth also occurred, they saw no reason to postulate methods of transmission different from those of other species of *Leishmania* from mammals. We concur with this position and feel that *Endotrypanum* also is transmitted by inoculation during feeding, rather than by the contaminative fecal method.

It is not known if flagellates in the cardia may be regurgitated during feeding, or what effect subsequent feedings may have upon their distribution in the gut of the sand fly.

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