

**TRYPANOSOMES OF THE LESSER ANTEATER, TAMANDUA
TETRADACTYLA, FROM PANAMA**

Bryce C. Walton and Octavio E. Sousa

TRYPANOSOMES OF THE LESSER ANTEATER, *TAMANDUA TETRADACTYLA*, FROM PANAMA

Bryce C. Walton* and Octavio E. Sousa

U. S. Army Medical Research Unit Component, Middle America Research Unit, Box 2011, Balboa Heights, C. Z., and Gorgas Memorial Laboratory, Box 2016, Balboa Heights, C. Z.

ABSTRACT: Seventeen anteaters (*Tamandua tetradactyla*) from Panama were examined by blood examination and culture for trypanosomes. Eight of these exhibited trypanosomes, four of which were dual infections with more than one species. *T. legeri*, a little-known species not previously reported from Panama, was found in five animals and established in vitro culture for the first time. Observations concerning the biology of this species, as well as its biometric characterization based on a much larger number of parasites than has heretofore been available, are reported. The most prevalent species was *T. rangeli*, found in six animals. One infection with a *T. cruzi*-like trypanosome was encountered.

In 1910, Mesnil and Brimont described *Trypanosoma legeri* from an anteater, *Tamandua tridactyla*, from French Guiana which was quite different from all other mammalian trypanosomes known at that time. Because of the broad form and highly convoluted undulating membrane it was said to resemble a bird trypanosome more than a mammalian parasite. In addition, it possessed a distinctive character in that the free flagellum terminated in a knob-like enlargement. This terminal granule stained a deep violet color with Giemsa's stain, and was said to be always present. The measurements given for this trypanosome were 42 to 45 μ in total length and 5 to 6.5 μ in width.

Published references to this parasite subsequent to the original description have been rare. In 1926, Strong et al. reported its occurrence in *Tamandua tetradactyla* in Brazil, and this observation was repeated by Deane (1961). Lainson (1965) found five infected *T. tetradactyla* in British Honduras. The only report of this parasite in a host other than an anteater is that of Trejos and Montero-Gei (1953) who identified as *T. legeri* a trypanosome encountered in the blood of a sloth, *Bradypus griseus*, from Costa Rica. Other than to extend the known geographic and host range, these investigators were able to contribute little to the knowledge of this parasite. The natural vector has remained unknown, and no success in infection of laboratory animals has been achieved, nor has it been established in culture, although Strong et al.

(1926), Deane (1961), and Lainson (1965) reported attempts to do so.

Two other species of trypanosomes have been described from similar hosts: *T. myrmecophagae* from *Tamandua tridactyla* in French Guiana by Floch et al. (1941, 1949) and *T. mesnil-brimonti* from the sloth *Choloepus didactylus* in Brazil by Deane (1961).

From 1963 to 1965 we have observed this trypanosome in the blood of five *Tamandua tetradactyla* from Panama and successfully established it in in vitro culture. A preliminary report of some of these observations was given at the Third Latin American Congress of Microbiology (Walton and Sousa, 1964). This report will present additional information regarding its distribution and prevalence in Panama, observations regarding its biology, and a biometric characterization of this species based upon a much larger number of parasites than has heretofore been available.

MATERIALS AND METHODS

A total of 17 adult anteaters were examined by use of fresh blood films and inoculation of culture media. Most of these were livetrapped and bled by cardiac puncture under anesthesia; however, two which had been killed by automobiles on the highway were bled shortly after death. An additional two animals killed in this manner had the thorax crushed so that uncontaminated cultures could not be obtained and only blood smears were examined. The culture media used were blood-agar base (Difco) with 20% defibrinated rabbit blood, with 20% outdated human blood-bank blood, with 10% oxalated sheep blood, and a modified Leishmania Agar medium of Senekjic (Kirby 1950) prepared with 15%, 20%, and 30% rabbit blood.

Most cultures were incubated through 28 days

Received for publication 5 May 1967.

* Lieutenant Colonel MSC USA.

TABLE I. *Trypanosomes encountered by direct microscopic examination and in vitro culture of blood of Tamandua tetradactyla.*

Ident. no.	Locality	Direct	Culture	Trypanosome sp.
1	Laguna, Darien, R. P.	—	— (14 days)	—
2	France Field, C. Z.	+	+	<i>T. legeri</i> and <i>T. rangeli</i>
3	France Field, C. Z.	+	+	<i>T. legeri</i> and <i>T. rangeli</i>
4	Chepo, R. P.	+	+	<i>T. legeri</i>
5	Los Rios, C. Z.	—	+	<i>T. rangeli</i>
6	Cabo Verde, R. P.	—	—	—
7	La Arena, R. P.	—	—	—
8	Pacora, R. P.	+	+	<i>T. legeri</i> and <i>T. cruzi</i> -like
9	Chiva Chiva, R. P.	—	+	<i>T. rangeli</i>
10	Ft. Sherman, C. Z.	+	+	<i>T. rangeli</i>
11	Summit Radio Sta., C. Z.	—	—	—
12	France Field, C. Z.	—	—	—
13	Cardenas Rd., C. Z.	—	Not done	—
14	Piña Rd., Ft. Sherman, C. Z.	—	Not done	—
15	Ft. Davis, C. Z.	—	—	—
16	Bique, R. P.	—	— (14 days)	—
17	Bique, R. P.	+	+	<i>T. legeri</i> and <i>T. rangeli</i>

at 25 C, although with two animals the tubes were discarded when no growth was observed at 14 days. Whole blood, in which trypanosomes were seen on direct examination, was inoculated intraperitoneally or subcutaneously into young adult CFW and C:HI mice, laboratory rats, a silky anteater (*Cyclopes didactylus*), and hamsters. Mice, rats, and hamsters were similarly inoculated with culture forms from 8-day-old cultures. Blood films from infected anteaters and inoculated laboratory animals were stained with Giemsa's stain for morphologic studies. Measurements were obtained with the aid of a camera lucida and a pair of dividers. All measurements are given in microns.

RESULTS

Trypanosomes were encountered in blood, cultures, or both, in eight of the 17 anteaters from localities on both coasts of the isthmus (Table I). A strong subjective impression of two types of parasites occurring in the same animal was formed after examining fresh blood preparations from three of the animals. Study of stained smears, culture results, and mouse inoculation experiments indicated that there were mixed infections in four anteaters.

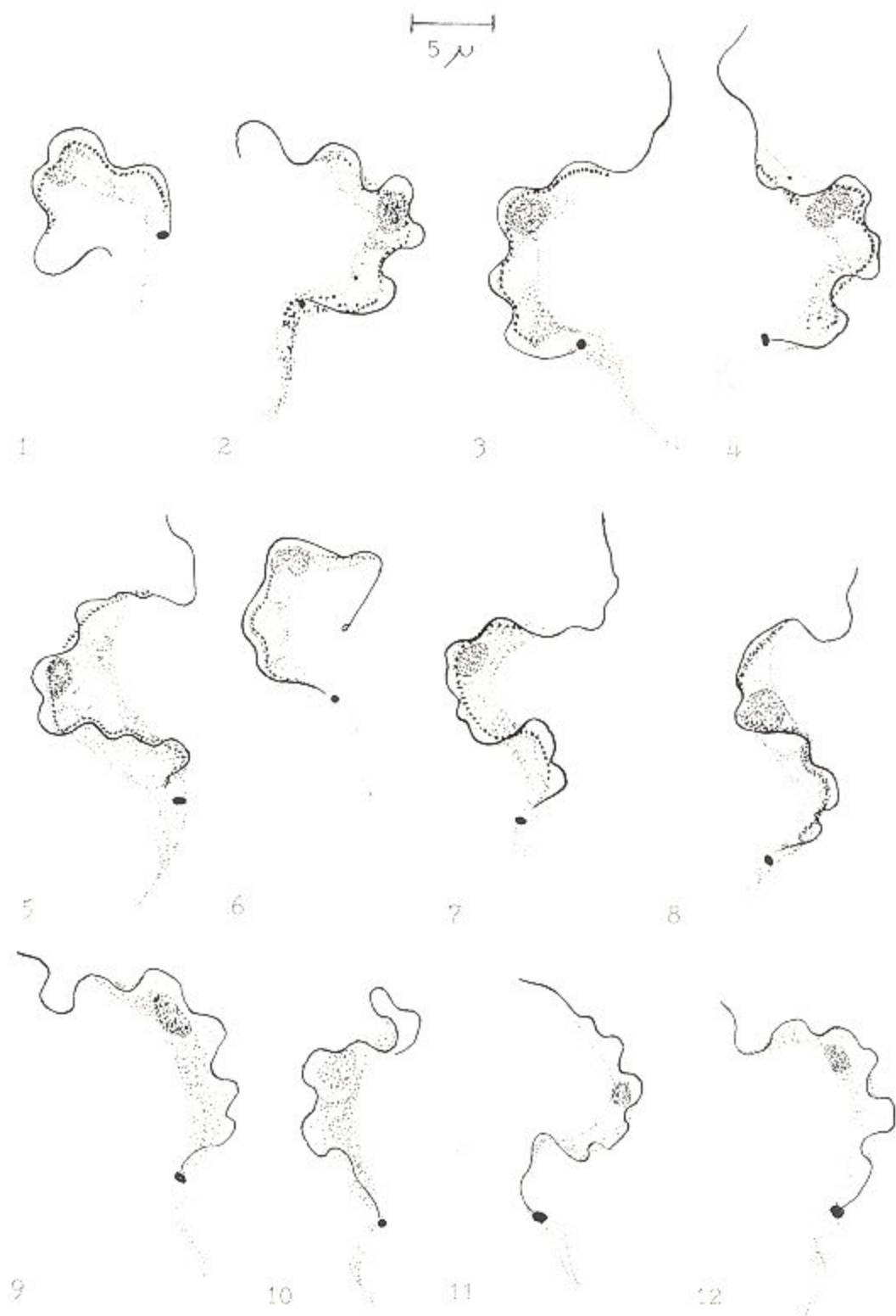
Morphology

Stained blood films from animals 2, 3, and 17 showed mixed populations of trypanosomes; slender forms whose appearance and measurements were compatible with those of *T. rangeli*, *T. myrmecophagae*, or *T. mesnil-brimonti*, as well as forms exhibiting all of the distinctive characteristics of *T. legeri*. The latter type (Figs. 1-8) were quite plentiful, and had the triangular-shaped postkinetoplast projection, the highly convoluted undulating membrane, and many had the terminal knob on the free

flagellum. The vacuole immediately posterior to the nucleus as described by Deane and Lainson was almost invariably present. The kinetoplast was often rod-shaped and marginally located, varying in distance from the posterior end of the body. In the larger forms a vacuole was also found anterior to the kinetoplast. Volutin granules were frequently seen in the cytoplasm, at times concentrating towards the posterior third of the flagellate, or forming a continuous row of large granules at the base of the undulating membrane. Such a row of volutin granules was very prominent in some of the specimens (Figs. 1-8).

Trypanosomes were very numerous in the blood of the anteaters 2 and 3, collected from France Field in the Canal Zone. A biometric study of 125 trypanosomes from these two animals (Table II) revealed a wide range of variation in total length (20.2 to 48.4). Some of the smaller trypanosomes were relatively broad, up to 4.8 in width while the longer trypanosomes were generally more slender. Two populations of trypanosomes could be detected (Table II, Fig. 13). Type I was shorter, usually broader, with an anteriorly located nucleus (nuclear index 1.5 or more), and a shorter free flagellum (less than 10) and usually exhibited the characteristics of *T. legeri*. Type II was longer (up to 48.4), more slender, with a more posteriorly situated nucleus (nuclear index less than 1.5), and a free flagellum often longer than 10. These trypanosomes lacked volutin granules and resembled *T. rangeli*.

The trypanosomes found in anteater 4 were



FIGURES 1-12. Trypanosomes of the lesser anteater, *Tamandua tetradactyla*. 1-4. *T. legeri* in blood films of anteater 2. 5-8. *T. legeri* in blood films of anteater 3. 9-12. *T. rangeli* in blood films of anteater 5.

TABLE II. *Biometric study of 125 trypanosomes found in the blood of two Tamandua tetradactyla from Panama.*

	Trypanosomes	
	Type I (broad form)	Type II (slender form)
No. measured	80	45
Nuclear Index (PN/NA)	1.5	1.5
Total length	20.2-40.7 (31.5)	31.6-48.4 (41.8)
Body length	17.2-31.7 (24.6)	21.6-38.4 (30.1)
Width	1.6-4.8 (2.7)	1.6-3.8 (2.2)
PK	2.5-10.0 (5.8)	2.6-10.5 (5.9)
K	0.4-1.0 (0.7)	0.4-1.0 (0.7)
KN	6.7-13.5 (10.3)	5.1-11.0 (8.5)
NA	2.6-10.7 (6.7)	8.9-20.4 (14.9)
Free flagellum	3.0-11.1 (7.7)	9.0-14.4 (11.6)

PK—Posterior end to kinetoplast.
KN—Kinetoplast to middle of nucleus.
NA—Middle of nucleus to anterior end.
PN—Middle of nucleus to posterior end.

of only the *T. legeri* type. Their measurements (Table III) were compatible with the shorter Type I trypanosomes of the mixed infection, and had an anteriorly located nucleus (nuclear index 1.5), and short free flagellum. This animal was maintained in the laboratory for a period of 1 year. Periodic blood examinations during this time failed to reveal any of the large, slender Type II trypanosomes. Repeated cultures and xenodiagnostic procedures excluded any possibility of a mixed infection with either *T. cruzi* or *T. rangeli*. The morphology and measurements of the trypanosomes in this animal remained constant and compatible with *T. legeri*.

In vitro culture

Poor growth was obtained with blood-agar base media containing 20% human blood, or 10% sheep blood with anteaters 2 and 3. Few organisms were seen and none survived subpassage. This medium containing 20% rabbit blood yielded abundant organisms with inocula from the same two animals. Stained smears revealed some broad crithidia with triangular beaklike posterior projections resembling the

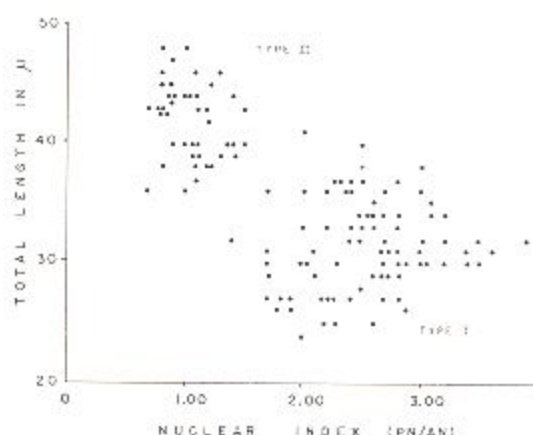


FIGURE 13. Measurements of 125 trypanosomes in mixed infections from *Tamandua tetradactyla* from the Canal Zone.

broad trypanosome in blood, although the great majority were long and slender. After two or three subpassages only slender crithidia were seen, which tended to be even more elongate than the original isolates. In retrospect, it would appear that both the original isolates contained two parasites, and the broader *T. legeri* was lost in subpassage. Culture tubes inoculated with blood from anteater 4, in which only *T. legeri* was identified, yielded only scanty organisms even in 20% rabbit blood medium. In Senekje's medium containing 30% rabbit blood, the strain has survived over 30 in vitro passages although the number of organisms always remained at low levels. The crithidial forms in culture exhibit many characteristics of the blood-stream forms (Fig. 14). Cultures inoculated with blood from anteater 5, which exhibited only the *T. rangeli* type trypanosome, yielded abundant growth of the slender type crithidia in all of the rabbit blood media. Tubes inoculated with blood of anteater 8, which showed only *T. legeri*, supported growth which

TABLE III. *Measurements of the trypanosomes in the blood of the anteater, Tamandua tetradactyla, from Panama.*

Animal no.	Mixed infections		<i>T. legeri</i>
	2	3	4
Specimens measured	55	70	15
Total length	24.1-43.9 (34.6)	20.2-48.4 (35.4)	24.1-36.5 (30.0)
Body length	17.2-32.6 (25.4)	18.3-38.4 (26.3)	19.1-28.9 (24.4)
Width	1.6-4.1 (2.4)	1.6-4.8 (2.6)	1.9-3.2 (2.4)
Flagellum	4.1-13.6 (9.1)	4.3-14.0 (9.1)	3.7-9.0 (5.6)
Nuclear index	0.98-3.60 (2.20)	0.67-5.23 (1.94)	1.54-3.48 (2.67)

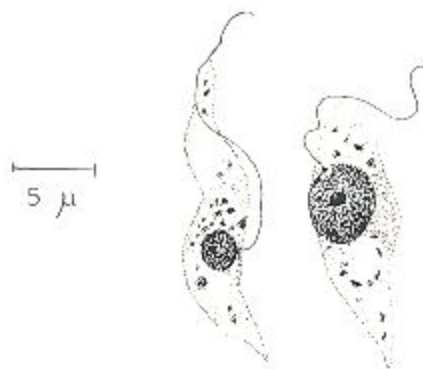


FIGURE 14. Crithidia of *T. legeri* from culture obtained from anteater 4.

eventually provided two culture isolates, a strain of *T. legeri*, and a strain of *cruzi*-like crithidia.

Animal inoculations

No parasitemia was observed in mice, rats, hamsters, and a silky anteater (*Cyclopes didactylus*) inoculated with blood of anteaters 2 and 3. Mice inoculated with 8-day cultures from these anteaters, which showed both broad culture forms thought to be *T. legeri* and slender forms, produced a parasitemia beginning at 24 hr. The numbers steadily increased through about 14 days when they began to diminish. The infection appeared to be benign and no mortality occurred. Stained smears revealed all trypanosomes were slender forms resembling *T. rangeli* and no forms exhibiting characters of *T. legeri* were ever observed. Rats and hamsters inoculated with later in vitro subpassages of these strains after the broad forms were no longer seen, showed transient parasitemias with the same character. Inoculation of mice with cultures from anteater 5 yielded similar results. In several attempts, no success was encountered in establishing serial syringe passages in mice by inoculation of blood from those infected with culture forms.

DISCUSSION

The slender trypanosomes encountered in these Panamanian anteaters were morphologically indistinguishable from *T. rangeli*, which is frequently encountered in a variety of other hosts in the area. For this reason, they are identified here as *T. rangeli*, but the question of the application of the name *T. myrmecoph-*

agae or *T. mesnil-brimonti* has not been considered in this study since the status of these species is not entirely clear.

The terminal granule at the extremity of the free flagellum was described by Mesnil and Brimont (1910) as a constant feature of *T. legeri*. Trejos and Montero-Gei (1953) found it on the three organisms they describe; and Deane (1961) lists it as a character in his redescription of the species. However, Lainson (1965) did not mention this structure in his account of the parasite from British Honduras, nor did he indicate its presence on any of the six organisms in his figures. In our stained blood films, it is apparent in only about 10% of the trypanosomes exhibiting other characters of *T. legeri*. The anterior position of the nucleus, the vacuoles near the nucleus and kinetoplast, the chain of large volutin granules, and the triangular post-blepharoplast projections, all appear to be much more consistent distinctive characters of the species.

The reference to *Trypanosoma legeri* in Marcel Leger's note to C. Franca (Leger, 1912) is obviously a *lapsus calami*. As indicated by the context, and reference to an avian host, the intended name was *Leucocytozoon legeri*. However, since this note was referenced in the Index-Catalog of Medical and Veterinary Zoology, we would like to call attention to this erroneous citation.

LITERATURE CITED

- DEANE, L. M. 1961. Tripanosomídeos de Mamíferos da Região Amazônica. I. Alguns flagelados encontrados no sangue de mamíferos silvestres do Estado do Pará. *Rev. Inst. Med. Trop. São Paulo* 3: 15-28.
- FLOCH, H., P. LAJUBE, AND E. ABONNENC. 1941. Un trypanosome d'un édenté (*Myrmecophaga tridactyla*) susceptible d'évoluer dans le tube digestif de *Rhodnius prolixus*. *Inst. Pasteur Guyane. Fr. Terr. Unini Publ.* no. 25: 1-6.
- , AND E. ABONNENC. 1949. Trypanosomes des mammifères silvestres, autres que *S. cruzi*, en Guyane Française. *Inst. Pasteur Guyane Fr. Terr. Unini Publ.* no. 193: 1-14.
- KIRBY, H. 1950. *Materials and Methods in the Study of Protozoa*. Univ. California Press, Berkeley and Los Angeles, p. 19.
- LAINSON, R. 1965. Parasitological studies in British Honduras. I. A parasite resembling *Trypanosoma* (*Schizotrypanum*) *cruzi* in the coatí, *Nasua narica* (Carnivora, Procyonidae), and a note on *Trypanosoma legeri* from the anteater, *Tamandua tetradactyla* (Edentata). *Ann. Trop. Med. & Parasit.* 59: 37-42.

- LEGER, M. 1912. (Note to C. Franca). Bull. Soc. Path. Exot. **5**: 10.
- MESNIL, F., AND E. BRIMONT. 1910. Trypanosome et microfilaire d'un édenté, le *Tamandua tridactyla* (L.). Compt. Rend. Soc. Biol. **69**: 148-151.
- STRONG, R. P., G. C. SHATTUCK, J. C. BEQUAERT, AND R. E. WHEELER. 1926. Medical Report of the Hamilton Rice Seventh Expedition to the Amazon, in Conjunction with the Department of Tropical Medicine of Harvard University, 1924-1925. Harvard University Press, Cambridge, p. 107-111.
- TREJOS, A., AND F. MONTERO-GEI. 1953. Estudio sobre tripanosómidos de Edentata en Costa Rica. I. *Trypanosoma legeri* en *Bradypus griseus*. Rev. Biol. Trop. **1**: 21-27.
- WALTON, B. C., AND O. SOUSA. 1964. Report of the Occurrence of *Trypanosoma legeri* in *Tamandua tetradactyla* in Panama. Tercer Cong. Latino-Amer. de Microbiol., Bogotá, Dec. 1964.