

Trypanosoma thecadactyli sp. n. from Forest Geckoes in Panama, and its Development in the Sandfly *Lutzomyia trinidadensis* (Newstead) (Diptera, Psychodidae)*

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SYNOPSIS. *Trypanosoma thecadactyli* sp. n. is described from the forest gecko *Thecadactylus rapicaudus* in Panama. Blood smears from 18 of 25 (72%) geckoes sampled were positive for the new trypanosome. The flagellate, ~22 μ m long, is ovoid or roughly triangular in shape and has a posterior cytoplasmic projection. Tree buttresses, rock crevices, and caves are microhabitats shared by *T. rapicaudus* and the phlebotomine sandfly *Lutzomyia trinidadensis*. Approximately 20% of the wild-caught and dissected *L. trinidadensis* were infected with flagellates. Twenty-four of 45 (53%) laboratory-reared *L. trinidadensis* allowed to feed on parasitized geckoes developed heavy infections of the mid- and hindgut which persisted up to 2 weeks, the longest postprandial period before dissection. Transmission probably occurs as a result of geckoes feeding on infected sandflies in nature. The distribution of *L. trinidadensis*, from Mexico to Brazil, approximates that of the gecko. Laboratory results and ecologic observations implicate *L. trinidadensis* as a potential vector of *T. thecadactyli*.

Index Key Words: *Trypanosoma thecadactyli* sp. n.; forest gecko, *Thecadactylus rapicaudus*, host of; sandfly, *Lutzomyia trinidadensis*, vector of; taxonomy; life cycle.

TRYPANOSOMES of lizards have been recorded from many parts of the world (4, 19), but potential vector relationships have been described for only a few species. *Trypanosoma phlebotomi* (Mackie) and *Trypanosoma platyductyli* Catouillard, parasitic in the Old World geckoes *Hemidactylus frenatus* Dumeril & Bibron and *Tarentola mauritanica* Gray respectively, were reported to develop in phlebotomine sandflies (1, 17). Recently, Ayala (3) reported extrinsic development of *Trypanosoma gerthionoti* Ayala & McKay and *Trypanosoma scelopori* Ayala of the California lizards *Gerthionotus multicarinatus* (Blainville) and *Sceloporus occidentalis*, (Baird & Girard) respectively, in the phlebotomine *Lutzomyia vexator occidentalis* (Fairchild & Hertig).

The high flagellate infection rates among Panamanian sandflies have been documented by workers at Gorgas Memorial Laboratory. Trypanosomatid genera infecting *Lutzomyia* in this country include *Leishmania* (7, 11, 12, 14), *Trypanosoma* (8, 13), *Endotrypanum* (16), and possibly *Crithidia* (18). To understand the extent to which various species of sandflies are involved in the transmission of *Leishmania*, it is helpful to clarify their involvement with related trypanosomatid genera.

Trypanosomes were found in the majority of forest geckoes, *Thecadactylus rapicaudus* (Houttuyn), examined during an ecologic survey for leishmaniasis in 1968 and 1969. According to concurrent studies of Panamanian sandflies there was a high correlation between the preferred microhabitats of geckoes (tree buttresses, rock crevices, and limestone caves), and the resting sites of the sandfly *Lutzomyia trinidadensis* (Newstead). A series of experimental feedings by laboratory-reared *L. trinidadensis* on infected *T. rapicaudus* was begun in October 1968, to study the vector potential of this sandfly species.

MATERIALS AND METHODS

Geckoes were collected by hand or noose, usually while searching tree buttresses, old buildings, rock crevices or caves for sandflies. Within a day after arrival of the lizards at the laboratory, blood smears obtained from a toe or brachial artery

were fixed in methanol and stained with Giemsa's stain. Subsequent blood smears were made routinely at ~5 day intervals. Most geckoes survived for several months in screened cages on a diet of meal worms and mosquitoes.

Sandflies were collected with battery or mouth operated aspirators from the microhabitats mentioned above. *L. trinidadensis* larvae were reared by the technic of Hertig & Johnson (10). Adult flies were maintained and fed within specially constructed plastic take-cover cages (6) on unrestrained infected geckoes.

Dissections of wild-caught *L. trinidadensis* were made shortly after capture. Laboratory-reared flies of generations F₁ to F₅ were dissected at different periods after feeding on infected *T. rapicaudus*. The infected guts of many dissected flies were triturated individually and inoculated into Senekji's agar slants and Noguchi's semisolid agar. Approximately 0.5 ml of an antibiotic mixture of penicillin and streptomycin 500 un/ml, and 1 mg/ml, respectively, was added as an overlay to each tube of culture media.

RESULTS

The trypanosome which parasitizes *T. rapicaudus* in Panama is clearly distinct from all other trypanosomes described from New World lizards, and accordingly we designate it:

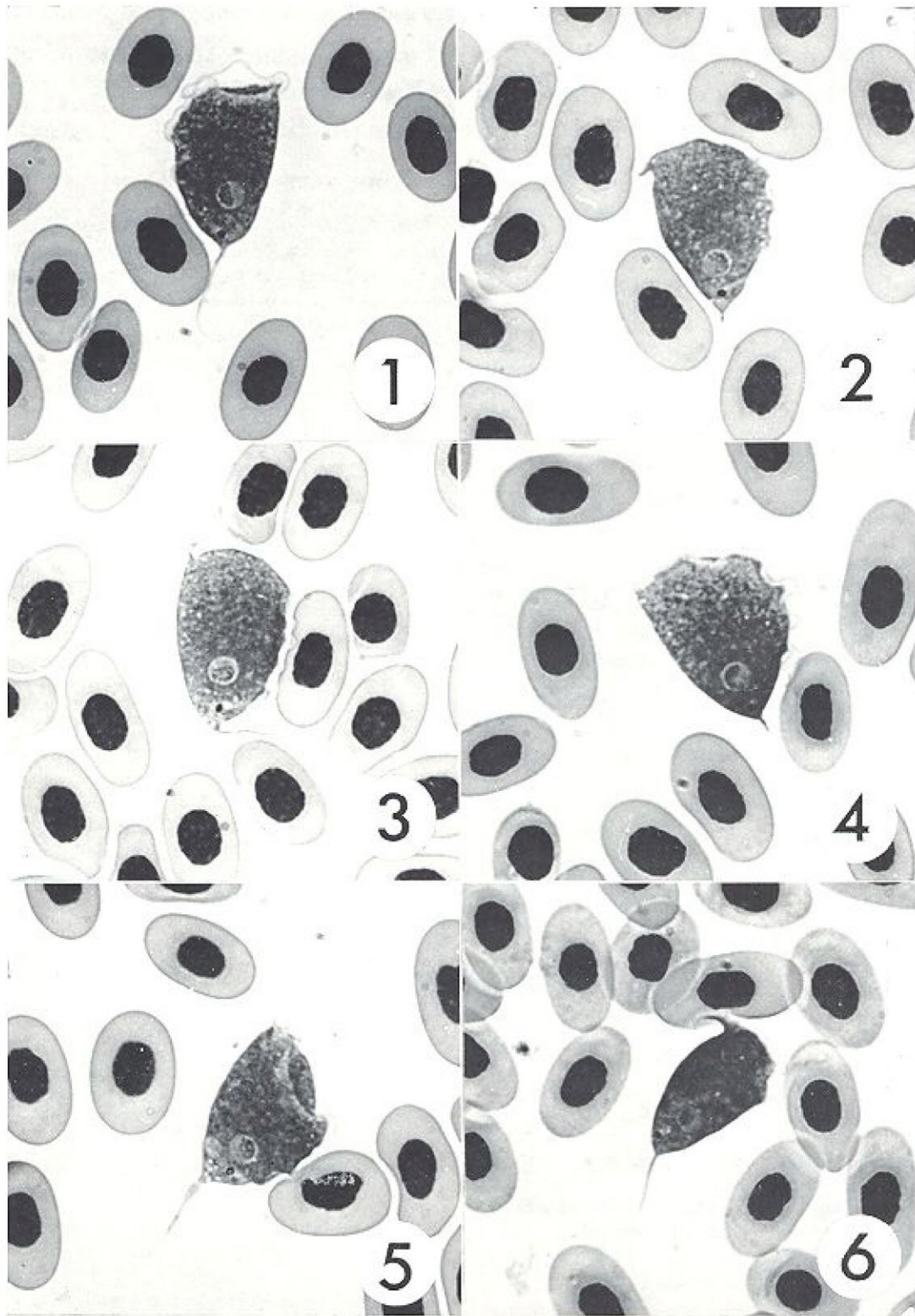
Trypanosoma thecadactyli sp. n.

(Figs. 1-6)

Diagnosis (all measurements in μ m, based on 20 determinations).—Monomorphic; body ovoid or roughly triangular, occasionally elongate, measuring 21.9 (19-24) in length (excluding anterior and posterior projections) and 9.1 (6.5-12.5) in width (at level of nucleus); with prominent posterior cytoplasmic projection, 9.3 (2-15), extending ~10-60% of body length; short anterior projection, 1.7 (0-4.5) occasionally present; flagellum attached by undulating membrane along 1/2-4/5 body length; free flagellum 12.4 (9-20) long; nucleus with deep-staining, eccentric chromatin mass giving appearance of half moon, surrounded by clear perinuclear area; cytoplasm strongly basophilic in Giemsa's stain; distance from posterior body end to kinetoplast center (excluding posterior projection), 2 (1-4); distance from kinetoplast center to center of nucleus,

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Figs. 1-6. [*Trypanosoma thecadaetyli* sp. n. in Giemsa's stained blood smears from the forest gecko *Thecadaetylus rapicaudus*. All figures, $\times 1,000$.] 1-4. Characteristic half moon appearance of nucleus. 5. Constant, prominent posterior cytoplasmic projection. 6. Occasionally present anterior cytoplasmic projection.

4.2 (3.5-5) and that from center of nucleus to anterior body end (excluding anterior projection), 14.4 (11-16.5).

Type Host.—*Thecadaetylus rapicaudus* (Houttuyn). Chagres Boy Scout Camp, Madden Forest, Canal Zone, Republic of

Panama. Syntype slide in Telford collection; parasyntype slides in Department of Leishmaniasis, Gorgas Memorial Laboratory, Panama, and in Department of Zoology, University of California at Los Angeles.

Remarks

T. thecadactyli is known at present only from the Canal Zone, adjacent Panama and Colon provinces, and San Blas Territory, Republic of Panama. The new species was found in 18 of 25 (72%) *T. rapicaudus* examined, all infected hosts having low-grade parasitemias.

T. thecadactyli is the only described saurian trypanosome with a constant distinct posterior cytoplasmic projection. It may be distinguished from *T. ocumarensis*, described by Scorza & Dagert (15) from a Venezuelan *Thecadactylus rapicaudus*, by its smaller size (a mean length of 21.9 μ m in contrast to 42.5 μ m for *T. ocumarensis*) and by the half moon appearance of its nucleus in contrast to the pyriform nucleus in the Venezuelan species.

Invertebrate host

Flagellates were observed in the digestive tract of 18 of 88 (20%) wild-caught female *Lutzomyia trinidadensis* collected from 4 localities in the Republic of Panama during 1968 and 1969. Hindgut infections were found in all positive sandflies; however, flagellates were present in the midgut of 4 flies (22%), 2 of which also had infections of the cardia. Further, the parasites were found in the Malpighian tubules of 2 flies (11%). The rectal ampulla and portions of the hindgut commonly were enlarged as a result of massive infections.

During a 1 year period, 45 laboratory-reared *L. trinidadensis* were examined 1-14 days after feeding on geckoes infected with *T. thecadactyli*. Flagellates were observed in 24 of the flies (53% infection rate) following the 3rd-14th postprandial day. The parasites occasionally persisted in the midgut through the 7th postprandial day, but they also invaded the pylorus, hindgut, and rectal ampulla, with a fairly even distribution throughout the alimentary tract posterior to the cardia. After digestion of the blood-meal ~2% of the infections were restricted to the hindgut. The majority of trypanosomes within the sandfly gut were epimastigote forms; however, a few trypomastigotes were seen in most infections. The pylorus and other areas of the hindgut often were distended with massive concentrations of flagellates.

T. rapicaudus LR-1, collected on 7 July 1968 from Quebrada Bonita Cave, Colon Province, had *T. thecadactyli* in blood smears at capture. This animal remained patent until 22 March 1969, after which time blood smears were consistently negative. Fifteen laboratory-reared *L. trinidadensis* were fed on this gecko on different occasions from 7 June 1969-25 August 1969 and were examined 5-9 days after feeding. All flies were negative for flagellates.

Two lots of laboratory-reared *Culex pipiens quinquefasciatus* Say, totaling 22 females fed on infected *Thecadactylus*, were examined 1-11 days later. All mosquitoes were negative for flagellates.

Flagellate-free *Gonatodes albugularis fuscus* (Hollowell), a gekkonid relative of *Thecadactylus*, were collected within the city of Panama for transmission studies. One of these animals was force-fed 9 colony *L. trinidadensis* which had fed on an infected *Thecadactylus* 7 days earlier. In addition, saline triturated guts from 4 heavily infected colony flies, as determined by dissection, were inoculated intraperitoneally into each of 2 *Gonatodes* from the above series. Blood smears were examined subsequently from the 3 animals every few days. All lizards remained negative for trypanosomes.

Trypanosomes from wild-caught *L. trinidadensis* failed to grow on Senckji's solid agar slants and Noguchi's semisolid agar. Trypanosomes isolated from colony flies after feedings on in-

fectured *Thecadactylus*, as well as those from heart blood from lizards, also failed to grow in these media.

DISCUSSION AND CONCLUSIONS

Shortt & Swaminath (17) infected laboratory bred *Phlebotomus babu* var. *shortti* (= *Sergentomyia babu shortti*) (Adler & Theodor) in India by feeding them on *Hemidactylus frenatus* parasitized by *Trypanosoma phlebotomi*. The infected flies, in which large number of epimastigote forms developed in the mid- and hindguts, were fed to clean geckoes which subsequently developed an infection.

Adler & Theodor (1) reported that *Trypanosoma platydactyli* development was restricted to the midgut and esophagus in *Phlebotomus parroti* (= *Sergentomyia minuta*) (Rondani) which had fed on infected *Tarentola mauritanica*. These authors, working in Malta, described transmission to a gecko which apparently acquired the trypanosome by swallowing an infected *S. minuta*. They did not, however, rule out the possibility that the infection may have resulted from the bite of the sandfly.

According to Chaniotis & Anderson (5), the trypanosome infections found in 4.6% of *Lutzomyia vexator occidentalis* in California probably were due to feeding on infected reptiles or anurans, the fly's preferred hosts. Ayala (2) observed 2 epimastigote flagellates in the stomach of a wild-caught *L. v. occidentalis* which had fed 10 days previously on a western fence lizard, *Sceloporus occidentalis*, infected experimentally with *Trypanosoma scelopori*. He later found the development patterns of *Trypanosoma gerrhonoti* and *T. scelopori* in *L. v. occidentalis* to be very similar, and suggested that perhaps the trypanosomes were conspecific (3). Epimastigote forms were found usually in the cardia, but occasionally in both esophagus and stomach. In contrast to the typical hindgut infections of *T. thecadactyli* in *L. trinidadensis* reported here, the California species localize in the midgut and cardia of *L. v. occidentalis*. This suggests a different mode of transmission from that of the Panamanian trypanosome.

Our experimental laboratory findings implicating *L. trinidadensis* as an intermediate host and potential vector for the forest gecko parasite *T. thecadactyli* are corroborated by the close ecologic association of the sandfly and lizard in nature. *L. trinidadensis* has a distribution from Mexico to Brazil (9), which approximates that of the gecko. *L. trinidadensis* is also the dominant sandfly species in tree buttresses in Panama, a favorite microhabitat of the gecko. McConnell & Correa (13) suggested that the trypanosome infections which they described in *L. trinidadensis* (3.3% infection rate) might have been acquired from feeding on lizard hosts of this species.

The characteristic posterior station infection in the sandflies indicates that transmission to the vertebrate host most likely takes place through the alimentary tract when the geckoes feed on infected flies. Many sandflies collected in tree buttresses are distended with blood or eggs. Such individuals are more reluctant than others to fly when disturbed. This may be caused by the great increase in body weight, a narcotizing effect of a large blood meal, or a combination of these 2 factors. Blood-engorged or gravid flies are much larger targets for predators than their unfed or nongravid companions and probably are subject to higher predation rates, thus enhancing the rate of transmission of the parasite. Although *T. rapicaudus* have not been reported to feed on sandflies in nature, they have been observed feeding on *L. trinidadensis* in the laboratory (6).

Trypanosoma thecadactyli probably is host specific for the

forest gecko, although there is some evidence to suggest that *L. trinidadensis* may act as an intermediate host for other trypanosomes. McConnell & Correa (13) reported that in 1 of 7 *L. trinidadensis* infected with trypanosomes the flagellates were found in a fresh blood meal from a mammal, as judged by the presence of non-nucleated red cells. This observation does not preclude the possibility that the infection may have been acquired during a previous blood meal on a gecko.

Our observation that 1 of the geckoes parasitized by *T. thecadactyli* eventually became free of the infection is inconsistent with the high prevalence of the infection in nature. It is quite possible, however, that frequent reinfection is a salient feature of the epidemiology of geckoimid trypanosomiasis in Panama.

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