

REPORT ON A FIELD COLLECTION OF DIPETALOGASTER MAXIMUS
(HEMIPTERA TRIATOMINAE) (UHLER, 1894)

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S U M M A R Y

Details of a field collection of *D. maximus* made at El Triunfo in Mexico are given. 185 Specimens were collected and 110 examined for flagellates which were present in 6 bugs. An attempt was made to characterise these flagellates. While *T. cruzi* is present in the area other species of the family Trypanosomidae may also be capable of infecting *Dipetalogaster maximus*. Some evidence suggestig *Trypanosoma rangeli* exists as a sylvatic infection in *D. maximus* is presented.

I N T R O D U C T I O N

Dipetalogaster maximus the largest blood sucking reduviid bug in the world is found South of La Paz on the Baja California Peninsula of Mexico. This small land area of barren uphill country with rocky outcrops is the only locality recorded for this species. RYCKMAN & RYCKMAN⁹ have listed details of the nine field collections made to date. This paper records details of a further collection which formed the basis of our present laboratory colony.

M A T E R I A L A N D M E T H O D S

The senior Author stayed at El Triunfo 53 kilometres South of La Paz (altitude 1,770 feet). Arriving at the town 6.30 p.m. on 21/2/1974 he demonstrated photographs of *D. maximus* and found lodging with a man who claimed to have been bitten frequently by the bug on the hills. With the help of various villagers the collection was made from 22/2/1974 to 24/2/74.

Bugs were maintained in waxed ice cream cartons with corrugated filter paper as resting sites. The labelled cartons were sealed and transported to Brasília.

In Brasília the number of living and dead bugs were counted and the instar noted. All living bugs were examined for rectal flagellates. Since bugs were for breeding, care was taken not to damage the genitalia. Forceps without teeth were used to apply gentle pressure to the rectal area to express faeces. Bugs in which no faeces could be obtained were separated and re-examined at two weekly intervals until a satisfactory result was achieved. First and second instars were fed and examined when they had achieved the 3rd instar. When flagellates were found they were inoculated into two 15gm male Swiss 44 mice and in NNN culture. The positive slide was stained with Giemsa and kept for reference. Wet films of tail blood of inoculated mice was examined at 14, 21 and 28 days. If no flagellates were seen, xenodiagnosis with five 5th ins *R. prolixus* was performed in each mouse and these bugs in turn were examined at 30 days for flagellates.

R E S U L T S

Colonies of *D. maximus* were scattered in the rock piles on the tops of high hills around El Triunfo. Some idea of their frequency can be obtained from the results of the first day. Starting at 7a.m. two men examined 27 rock

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piles turning over the rocks and dismantled 10 rodent nests. At 3p.m. at the 24th rock pile near a place where cattle rest, 15 small instars were recovered. As has been previously noted strenuous dismantling of the rock pile is not a good way to recover bugs⁹. The best way is to strip to ones underpants and to sit among the rocks. As they come to suck blood, they can be easily captured. The range in which they appreciate the presence of a blood meal is up to 6 metres. Using this technique 32 other rock piles were examined in the subsequent two days and 5 other habitats of *D. maximus* were discovered.

Table I shows the total number of bugs caught in the 3 day period.

T A B L E I

Total bug catch by instar and mortality during return journey to Brasília

Instars	I	II	III	IV	V	Adult	Totals
Alive	54	39	14	6	7	4	124
Dead	44	15	2	0	0	0	61
Totals	98	54	16	6	7	4	185

The prominence of young instars is notable. Due to the difficulty of obtaining a blood meal in such an isolated location, mortality of

young instars must be high. Several local hill men confirmed independently that large stages were more commonly seen in June, July and doubted if bugs could be found in February. The high mortality of young instars during the return journey may be related to the cold air conditioning of the aircraft.

Table II shows that in 110 bugs, flagellates were found in six (5.6%). We had varying success maintaining these isolates in the laboratory and briefly report the details below.

Isolate I met nearly all the criteria laid down by BARRETO² to identify it as *Trypanosoma cruzi*. Only one Trypanosome could be found in a stained film, and this had the large kinetoplast of *T. cruzi*. Despite treating mice with Dexamethasone or Methotrexate, or irradiating them with 500R before inoculation, we could never raise sufficient parasitaemia for biometric data. However the trypanosome readily infected *R. prolixus* and *T. infestans* and amastigotes could be demonstrated in the heart of infected mice. Culture forms could be readily obtained by seeding NNN with mouse blood. Cross protection was demonstrated to the Y strain of *T. cruzi* and haemagglutinating antibodies against *T. cruzi* developed in the peripheral blood of inoculated mice. This isolate we believe therefore to be *T. cruzi*.

T A B L E I I

Details of bugs found to harbor flagellates from field collection

Number of isolate	Instar of bug	Quantity of flagellates in bug intestine	Infected mice	Infected culture
I	4th instar	+++	Yes	No
II	3rd instar	++	No	No
III	Adult ♀	+++	No	No
IV	5 instar	±	No	No
V	3rd instar	++	Yes	No
VI	2nd instar	±	No	No

Isolate II was impossible to isolate in mice or culture. The bug containing this isolate was the only one caught in the field that contained a blood meal. This blood meal was sent on filter paper to Dr. P.F.L. Boreham in Ascot England for identification using precipitin tests. These were negative for rodent or reptile blood but identified a primate feed. Since there were no

monkeys in the area, this feed must have been human. However since it was a third instar bug, it cannot be concluded that the human feed was the source of the infection in the bug intestine.

Isolate III occurred under curious circumstances. One of the adults caught in the field was found dead and both intestine and haemo-

lymph were full of flagellates. Unfortunately the strain did not infect mice or cultures. Giemsa stained slides of the haemolymph showed long epimastigotes (more than $40\ \mu$ long). Their anterior and posterior extremities were finely pointed with a small, punctate juxtannuclear kinetoplast. Many haemocytes contained intracellular forms of the parasite. The morphology was similar to *Trypanosoma rangeli* in the haemocoel of its invertebrate host.

Isolate V strain was isolated in mice; but similar to strain one, the parasitaemia was very low and did not increase after dexamethasone. The parasite had a large kinetoplast suggesting *T. cruzi* and grew readily in the intestinal tract of *R. prolixus* and *T. infestans*. No amastigotes could be found in the mouse myocardium. Cross protection against the Y strain of *T. cruzi* was also noted. We think it likely that this was *T. cruzi*.

Isolates IV and VI consisted of single flagellates only visualised in saline preparations of bug faeces and the faeces failed to infect mice or culture.

DISCUSSION

This appears to be the largest field collection of this species yet made. Young instars were very active in the hot sun and appeared usually after 10 a.m. darting spider like over the hot rocks. At sunset they disappeared. The large number of first instars suggests that many do not find a blood meal and indeed very few sources seem to be available. RYCKMAN & RYCKMAN⁹ have suggested lizards are the main food source but the evidence is circumstantial. Only one lizard was seen during these field collections. The same Authors have stronger evidence that *D. maximus* feeds on the wood rat (*Neotoma* sp.). The human feed recorded from this collection is the first, but local people claim to have been frequently bitten by this bug. The observation by residents in the area that there is a seasonal increase in the bug population should be checked. The mortality among the young instars during their passage to Brasil suggests resistance to environmental extremes is less in the young stages. The presence of natural flagellate infections in these bugs has been previously observed. Mazzotti has recorded all of the original reports of *Dipetalogaster* infected with *T.*

*cruzi*⁹. He found a total of 6/72 (8.3%) infected a result similar to this collection. RYCKMAN & RYCKMAN⁹ suggested that future isolates should be carefully studied to determine if some lizard adapted Trypanosome is present in *D. maximus*. Unfortunately the studies of the six isolates that we made were incomplete due to the difficulty of laboratory maintenance. Isolates IV and VI were so scanty that this is not surprising. Isolate I and V however were probably *T. cruzi*. Isolate I was almost completely characterised and established beyond doubt the existence of *T. cruzi* in the area. Isolate V is incompletely characterised according to the criteria of BARRETO². However the question arises as to whether all these criteria are necessary. What other Trypanosome species would have the characteristics of isolate V is a relevant question. The origin of these strains of *T. cruzi* is unknown, but wood rats seem a likely possibility⁹. Although RYCKMAN⁸ reports experimental infection in lizards with *T. cruzi*, we have been unable to repeat these experiments. No reptile has ever been found naturally infected.

There are a number of reports of isolations of Mexican strains of *T. cruzi* (MAZZOTTI⁶; TAY et al.¹⁰) in various localities in Mexico. There was no difficulty in establishing some of these strains in mice and studying their behavior (TAY et al.¹¹; RAMIREZ et al.⁷). However MAZZOTTI⁶ had difficulty in establishing strains from *D. maximus* which led RYCKMAN & RYCKMAN⁹ to raise the possibility of a lizard adapted trypanosome. Our results fail to fully resolve this problem. Although *T. cruzi* is present in the area, there is evidence other flagellates are present as well. Studies in our laboratory have shown that *D. maximus* is readily infected with various *T. cruzi* strains and that this species has value in the procedure of xenodiagnosis (BARRETO et al.¹; CUBA et al.⁴). Longitudinal observations have shown *D. maximus* maintain an intestinal infection with a Peru strain of *T. cruzi* up to 503 days. (ALVARENGA & MARSDEN unpublished observations). Isolate II and III failed to infect mice. It is possible that isolate II was a lizard flagellate, but this is pure speculation. There is evidence on the basis of death of the bug, the presence of flagellates in the haemolymph and their morphology that isolate III was *T. rangeli*. Other indirect evi-

dence is available from unpublished experiments by CUBA (1977). *D. maximus* inoculated intracoelomically with strains Peru and Panama of *T. rangeli* had a high mortality with intense parasitism of the haemolymph as described by TOBIE¹² and CUBA³. Insects 7-10 days after inoculation showed this parasitism which continued up to 100 days after infection. Invasion of the salivary glands could not be demonstrated either by dissection or attempts to transmit *T. rangeli* to mice by bites of infected bugs.

Another difficulty in accepting on partial evidence that this organism was indeed *T. rangeli* is that it would be the first report not only for *D. maximus*, but also for Mexico (D. ALLESANDRO⁵). Further field work is required to clarify this problem.

RESUMO

Relato sobre uma coleta de *Dipetalogaster maximus*

Foram coletados 185 exemplares de *Dipetalogaster maximus* (Triatominae), em fevereiro de 1974, em El Triunfo, 40 quilômetros ao sul da cidade de La Paz, na Baixa Califórnia, México. Realizadas algumas observações sobre a ecologia dessa espécie, a amostra apresentou grande predominância de estágios jovens e apenas um triatomíneo continha sangue no tubo digestivo, o qual foi identificado como de primata. Em 110 triatomíneos examinados, seis estavam infectados com flagelados (5,4%) e dois que infectaram camundongos foram caracterizados como *T. cruzi*. Um "isolate" de flagelados foi identificado, morfológicamente, como *T. rangeli* e os três restantes não puderam ser identificados.

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