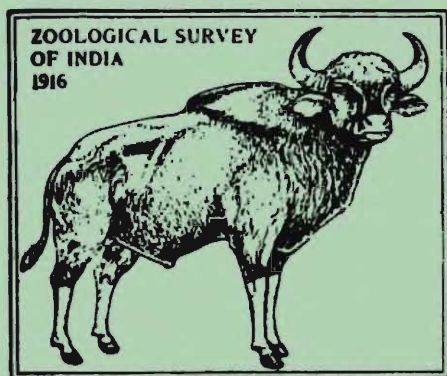


TRYPANOSOMES OF INDIAN ANURANS

by
R. RAY
A. CHOUDHURY



**ZOOLOGICAL SURVEY
OF INDIA**

Trypanosomes of Indian Anurans

TECHNICAL MONOGRAPH NO. 8

R. RAY

Zoological Survey of India, Calcutta

AND

A. CHOUDHURY

Department of Zoology, University of Calcutta



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INTRODUCTION

The trypanosomes of frogs and toads have been of interest to the parasitologist for many years, because they furnish excellent materials of academic interest. They also occupy important place in the evolution of trypanosomes because they present possibilities for the study of various fundamental phenomena relating not only to amphibian parasites but also those of mammals as well. As a result, there exists today a voluminous literature concerning anuran trypanosomes in the world (Baker, 1977; Bardsley and Harmsen, 1973, Diamond, 1958). Nevertheless, there is still much confusion as regards to the taxonomy of these parasites and little is known of their biology.

In India, the study on anuran trypanosomes was first initiated by Dr. N. Berestneff in 1903. He reported a trypanosome infection in *Rana tigrina* and *R. limnocharis*. After that, Patton (1908), Scott (1926, 1927), and Pujati (1953) contributed their knowledge to some extent on the literature of Indian anuran trypanosomes. Recently Ray (1979a, b, 1980), Ray and Nandi (1978), Ray and Choudhury (1981) studied the anuran trypanosomes in detail from India. All these publications had some inadequacies, so the necessity to have a comprehensive biological monograph on Trypanosomes of anuran amphibians of India was a long felt need. While describing each species all the synonymies have been given along with all the recorded references followed by type hosts, other host records, distribution, prevalence and morphology of the parasites in detail. The divisional and developmental stages of trypanosomes inside the vertebrate and invertebrate hosts are also included whenever it was available. The last three species of trypanosome described hereunder are new to science.

A key for the identification of species of Indian anuran trypanosomes has also been added.

The authors hope that this monograph on the trypanosomes of Indian anurans will stimulate more intensive and effective research in this field.

MATERIAL AND METHODS

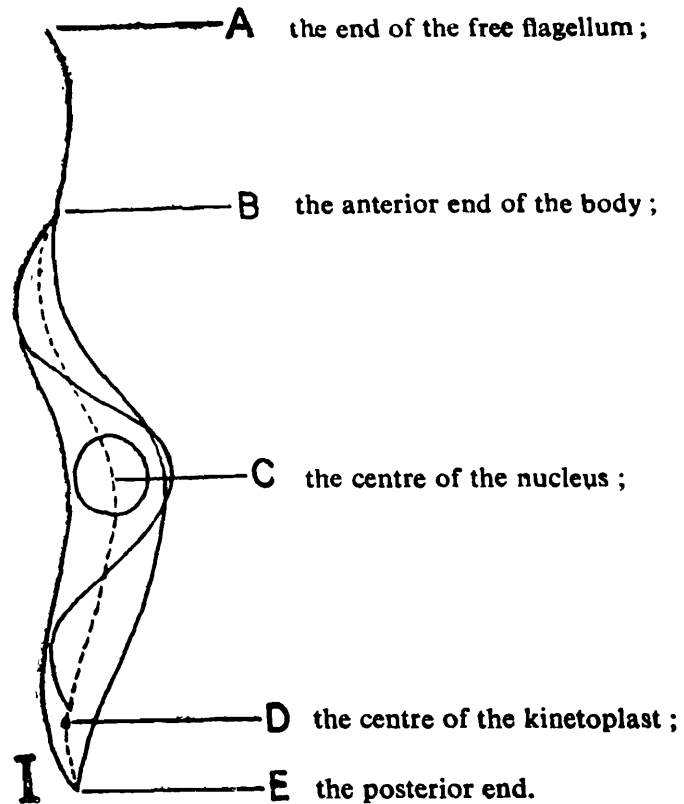
All the anuran amphibians were collected from different districts of West Bengal, Orissa, Bihar, Assam, Andaman Islands, Tripura, Andhra Pradesh, Kerala, Nova Goa etc., and examined for trypanosomes. Some amphibians collected from West Bengal, particularly from Bankura, Midnapore, Hooghly and 24-Parganas Districts, were brought to the laboratory and kept them alive for investigation.

Peripheral blood was obtained from the finger tips on alternate days at different intervals and was examined. Sometimes the infected hosts were autopsied and blood was taken directly from the heart by means of fine capillary pipette or hypodermic syringe. Impression and spread preparations were made from liver, lungs, kidney and bone-marrow. Air-dried blood films and organ smears were fixed in 100% methanol and stained with Romanowsky type of stains and examined.

Some gravid leeches like *Helobdella nociva*, *Hemiclepsis marginata* etc., collected from rural West Bengal were kept in finger bowls or glass vials containing aerated freshwater at room temperature. Young leeches hatched from the cocoons after 2/3 weeks were reared in the laboratory. Infected frogs (*Rana tigrina*) were fed by these young leeches for few minutes. After feeding the leeches were picked up gently and kept again separately in finger bowls with necessary markings.

The examination of the leeches was carried out at various set periods of time following Diamond's method (1958). For microscopical examination they were dissected after necessary narcotization in 4% aqueous Chlorobutanol (Pennak, 1953). The proboscis sheath, crops, salivary glands and crop punctate were examined. Citrated saline solution was also used for dilution of the crop or caecal content (3 : 1) to study the live specimens. Air-dried crop smears were fixed and stained with Romanowsky type of stains. The morphometric parameters were recorded after Diamond, 1958, Woo, 1969 and Hoare, 1972. The linear dimensions of the parasites were measured by drawing the image of trypanosome (Fig. I) by means of a camera-lucida on the drawing paper at a magnification of 1000 X. At least 25 individuals were measured. A line was drawn through the middle of the body from the posterior extremity to the tip of the flagellum and the important landmarks were then indicated with dots. Finally, the flagellum was drawn. Measurements were made with a pair of fine compasses the points of which are separated at a small unit measurements.

Twelve measurements were made from each trypanosome : the distance from posterior end to the center of Kinetoplast (PK), center of Kinetoplast to the centre of Nucleus (KN), from the posterior end to the center of the Nucleus (PN), center of nucleus to the anterior extremity (NA), the length of the body excluding free flagellum (PA), total length of the parasite including free-flagellum (TL), the length of free flagellum (FF), the maximum width excluding undulating membrane (BW), the length (L) and width (W) of the Nucleus and length and width of the Kinetoplast. The width of the undulating membrane was measured in some cases and number of undulation were also counted.



Useful indices were obtained by defining the position of the nucleus and kinetoplast. The nuclear index (NI) was obtained by the ratio of the distance from the later to the anterior end (PN/NA) (Dias and Freitas, 1943). When $NI = 1$, the nucleus is in the middle of the body, when it is < 1 , it is in the posterior part, and when it is > 1 , it is in the anterior part. The other kinetoplasmic index (KI) was arrived at dividing the distance from the kinetoplast to the nucleus (PK/KN). If KI is less than 2, the kinetoplast is nearer to the posterior end than to the nucleus, if it is 2, the kinetoplast is in half way between the two, if it is more than 2, the kinetoplast is closer to the nucleus (Keymer, 1967).

The positions of the various organelles in relation to each other in the trypanosomes were calculated by means of six ratios such as PK/PA, PN/PA, KN/PN, PK/PN, BW/PA and FF/PA.

In case of *Trypanosoma chattoni* which displays almost a rounded configuration, the body diameter and nuclear diameter were measured based on the average of two measurement taken at right angles to each other on each specimen. In addition to these, area measurement of the parasites is calculated by counting the number of squares covered (2 mm square in graph paper = $1 \mu m$).

The photomicrographs were taken with the help of "Ergavel C. Z. microscope" using PM 6 attachment camera.

KEY TO THE IDENTIFICATION OF ANURAN TRYPANOSOMES OF INDIA

- | | | |
|--|-----|---------------------------------|
| 1 (8). Body flattened into sheet-like form | ... | <i>Trypanosoma rotatorium</i> |
| 2 (3). Body in frontal view flattened but not sheet-like ; in lateral view widens anteriorly from kinetoplast, narrows posteriorly | ... | <i>Trypanosoma ranarum</i> |
| 3 (4). Body thick ; in frontal view compressed, ovoid, in lateral view elliptical, only about twice as long as wide | ... | <i>Trypanosoma loricatum</i> |
| 4 (5). Body elongate, striated ; extremities tapered ; kinetoplast located more than one-half distance from posterior extremity to nucleus ; absence of posterior vacuole or halo ; free flagellum less than the half of the body length | ... | <i>Trypanosoma karyozeukton</i> |
| 5 (4). Body elongate but not striated ; 'C' or 'S' shaped, kinetoplast located very close to the nucleus ; free flagellum is half the length of the body | ... | <i>Trypanosoma taprobanica</i> |
| 6 (5). There is a definite posterior halo or vacuole ; free flagellum is 1/3 the body length | ... | <i>Trypanosoma systoma</i> |
| 7 (4). Kinetoplast located less than one-half distance from posterior extremity to nucleus | ... | <i>Trypanosoma inopinatum</i> |
| The free flagellum is more than 3/4 of the body length | ... | <i>Trypanosoma malabarica</i> |
| 8 (1). Body spherical | ... | <i>Trypanosoma chattoni</i> |

GENERAL ORGANISATION

Genus *Trypanosoma* Gruby

Morphology : Parasite of the circulatory system of vertebrates ; usually slender and flattened, Pointed at flagellated end, and bluntly rounded or pointed at the other end ; usually pleomorphic, nucleus central ; near the flagellated end there is a blepharoblast from which the flagellum emerges and runs towards opposite end bordering the outer boundary of the undulating membrane ; in most cases the flagellum extends freely beyond the body as free

flagellum ; many with myoneme fibres ; multiplication by binary or multiple fission. The organism is transmitted from host to host by blood sucking invertebrates and undergoes a series of changes in the digestive system of the vector hosts. A number of these haemoflagellates are pathogenic to their hosts.

Life-cycle : Member of the genus *Trypanosoma* are mostly digenetic parasites i.e. the life-cycle of which alternates between two hosts : an invertebrate animal representing the intermediate host or vector (here leech), in which the flagellates develop primarily in the alimentary canal ; the other, a vertebrate animal (here frog), in which they inhabit the blood.

The genus *Trypanosoma* comprises :

a) trypomastigote b) amastigote c) promastigote d) epimastigote e) spheromastigote and f) metacyclic stages, while the trypomastigote being the typical form in the blood of the vertebrate hosts. Trypanosomes may assume any of the above six stages, which appear in different combination at various periods of their digenetic life-cycle, both the vertebrate and invertebrate hosts, according to the species of trypanosomes (Hoare, 1972).

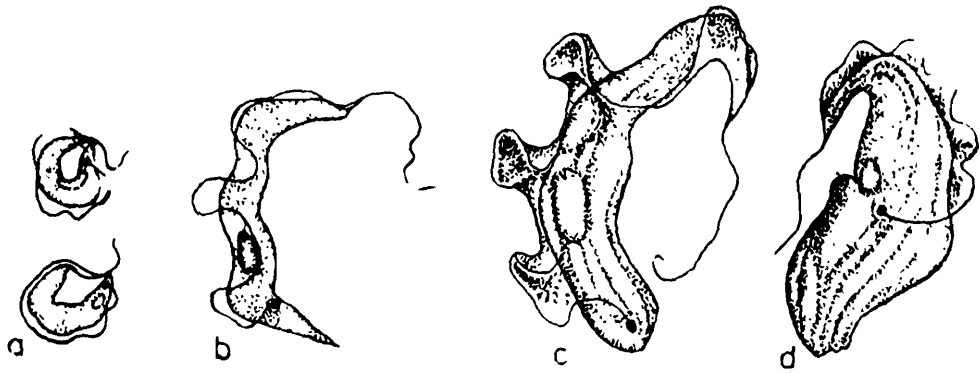
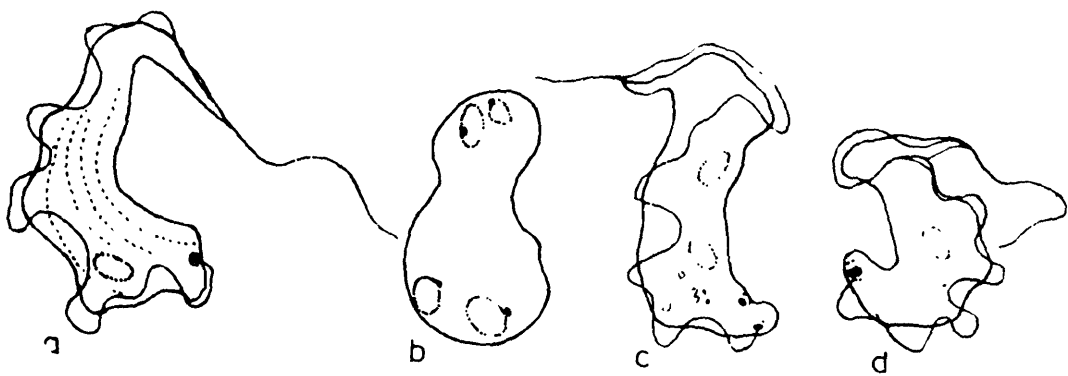
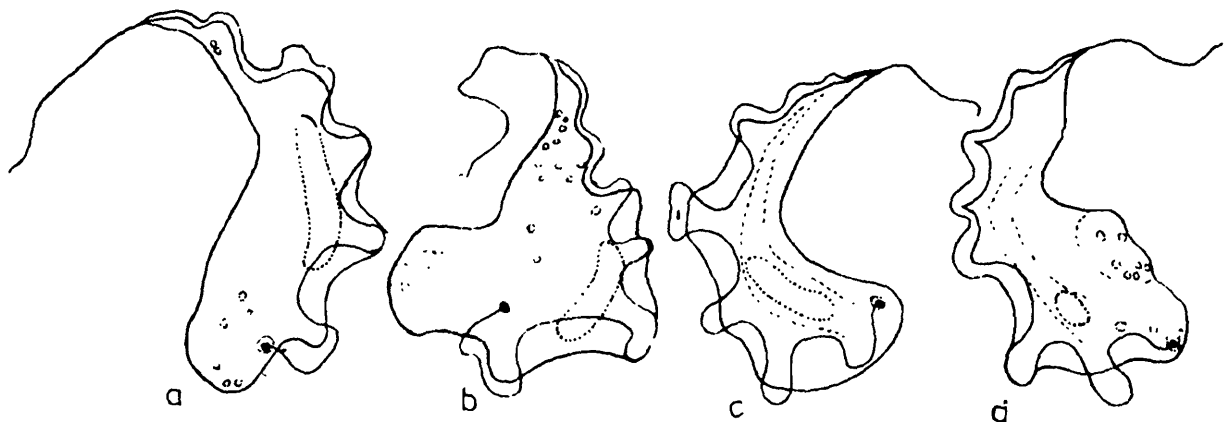
The trypanosomes live free in plasma of the vertebrate hosts and move actively in the blood with kinetoplast behind and free flagellum in front, like a snake wriggling backwards. They are pleomorphic, cosmopolitan and eryxenous, occurring in a wide range of hosts.

DESCRIPTION OF SPECIES OF TRYPANOSOMA

1. *Trypanosoma rotatorium* (Mayer)

(Figs. 1-6 and Plate I, II & III)

1843. *Amoeba rotatoria* Mayer, 'Spicilegium observationum anatomicarum de organo electrico in *Ralis anelicticis* et de Haematozois', Bonnæ : Chaussat, 1850, *Des hematozoaires* (Ph. D. Thesis, Paris).
1885. *Trypanomonas ranarum* Danilewsky, *Biol. Zbl*, 5 : 529 : 1889, La parasitologie comparee du Sang 1. Nouvelles recherches Sur les parasites du sang des Oiseaux, Kharkoff, Partim.
1885. *Trypanosoma ranarum* Danilewsky, *Ibid.* ; 1889, *Ibid.*
1885. *Trypanosoma sanguinis* Danilewsky, *Ibid.* ; 1889, *Ibid.*
1888. *Trypanosoma sanguinis ranarum* Shalashnikov, *Arkh. Vet. Nauk. S. Peterburg.*, 1 : 65.
1889. *Trypanomonodes ranarum* Danilewsky, La parasitologie comparee du sang 1. Nouvelles recherches Sur les parasites du sang des Oiseaux, Kharkoff, Partim.
1889. *Trypanosoma costatum* Danilewsky, *Ibid.*
1889. *Trypanosoma costatum ranarum* Danilewsky, *Ibid.*
1901. *Trypanosoma rotatorium* Lavarán and Mesnil, *C. r. séanc Soc. Biol. Paris*, 53 : 678 ; Wenyon, 1926, *Protozoology* Vol. 1., pp. 590 ; Pujati, 1953, *Doriana*, 1 : 1 ; Ray, 1979a, *Proc. 2nd Natl. Cong. Parasitol.*, p. 13, 1979b, 'Studies on the Haematozoa of Indian Amphibians, Ph. D. Thesis, Cal. Univ., 1980, *Indian J. Parasitology*, 3

FIG. 1 10 µmFIG. 2. 10 µmFIG. 3 10 µm

Figs. 1-3. Camera lucida drawings of *Trypanosoma rotatorium* from five anuran hosts.

Fig. 1. *T. rotatorium* in *Rana tigrina*. a, Type I (Juvenile form); b, Type II (Slender form); c, Type III (Flat leaf-like form); d, Type IV (Ledger compact form).

Fig. 2. *T. rotatorium* in *Rana limnocharis* and *Rana cyanophlyctis*. a, Trypomastigote stage in *R. limnocharis*; b, Dividing amastigote stage with 4 kinetoplasts and 4 nuclei in the same host; c, Dividing trypomastigote stage in *R. limnocharis*; d, Trypomastigote stage in *R. cyanophlyctis*.

Fig. 3. *T. rotatorium* in *Bufo melanostictus* and *Bufo stomaticus*. a & b, Typical leaf-like trypomastigote stage in *B. melanostictus*; c & d, The same in *B. stomaticus*.

- (Suppl.), : 87 ; Ray and Choudhury, 1980, *Proc. Sym. Host as an environment*, Z.S.I., p. 92, 1981, *Proc. 6th Int. Cong. Protozool.*, p. 308.
1907. *Trypanosoma loricatum* Dutton, Todd and Tobay, *Ann. trop. Med. Parasit.*, 1 : 287, Partim.
1908. *Trypanosoma* sp. Patton, *A Rep. King. Inst. Prev. Med. Guindy* (1907), 1 : 3 ; Berestneff, 1903, *Arch. Protistenk.*, 2 : 34 ; Scott, 1926, *Proc. zool. Soc. Lond.*, 1 : 231 ; 1927, *Proc. zool. Soc. Lond.*, 2 : 173 ; Weyon, 1926, *Protozoology*, Vol. 1, p. 588.
1908. *Trypanosoma hylae* Franca, *Archs R. Inst. Bact.*, 2 : 271, Partim.
- 1911a. *Trypanosoma borreli* Mathis and Léger, *C. r. Séanc. Soc. Biol.*, 70 : 956, Partim.

Type-host : *Rana esculenta* (Linn.).

Other hosts : *Rana tigrina*, *R. limnocharis*, *R. cyanophlyctis*, *Bufo melanostictus*.

New Indian hosts : *Bufo stomaticus*, *Rhacophorus maculatus* and *Rhacophorus malabaricus*.

Locality : Balitha and Kotalpur, Bankura district, Nakunda and Salikona, Hooghly district, Berhampur, Malda, Coochbehar and Jalpaiguri, West Bengal, Mollem, Volpoi and Bondla, Nova Goa ; Orissa ; Jamduar, Assam ; Tripura ; and Andaman Islands.

Prevalence : 51 out of 164 *Rana tigrina*, 37 out of 307 *R. limnocharis*, 11 out of 177 *Rhacophorus maculatus*, 3 out of 27 *R. malabaricus*, 25 out of 343 *Bufo melanostictus*, 5 out of 73 *B. stomaticus* and 5 out of 450 *Rana cyanophlyctis* were found positive for *Trypanosoma rotatorium*.

Description : *Trypanosoma rotatorium* obtained from the Indian Bull-frog, *Rana tigrina* Daudin is extremely pleomorphic (Wenyon, 1926 ; Nöller, 1913 ; Mohammed and Mansour, 1959). The following four forms were observed.

Type I [Juvenile form] [Fig. 1a & Plate I Fig. 1] :

The small 'C' shaped or 'S' shaped trypanoforms measure (PA) 13.83 μm (11.5-18.0 μm) in length and 2.26 μm (2.0-2.25 μm) in width. The posterior end is drawn out into narrow pointed extremity ; anterior end with a free flagellum, one third of the body length measuring 4.91 μm ; granulated cytoplasm stains light blue in Leishman and Giemsa. Cytoplasmic granules staining pink are homogeneously distributed throughout the body.

Nucleus, round or sometimes oval, stained purple with Leishman and Wright stains, is situated on the posterior part of the body (NI=0.27) in close association with the kinetoplast which is either rod-shaped or rounded

and surrounded by a halo. Kinetoplast stained deep blue, measured $0.66 \times 0.53 \mu\text{m}$ and attached with a parabasal body.

The flagellum, having its origin in the posterior extremity of the body from the kinetoplast, traverses the whole body, forming an undulating membrane ($1.2 \mu\text{m}$ in width), throwing 3-4 folds, ultimately leaving the anterior end as a free flagellum.

Type II (Slender forms) [Fig. 1b & Plate I Fig 2] :

This form is slender and elongated with attenuated posterior end measuring $23.5 \mu\text{m}$ ($23.2-24.0 \mu\text{m}$) in length and ($3.0-3.2 \mu\text{m}$) in width. Granulated cytoplasm stained blue with Giemsa, few granules arranged linearly at the anterior half but scattered at the posterior half of the body. Oval nucleus, staining purple in Leishman or Giemsa, situated at the posterior half of the body ($NI=0.4$). Kinetoplast is round or rod-shaped, situated at the attenuated posterior end ($KI=1.8$). The flagellum emerging from the side of the kinetoplast makes 3-5 attachments with the cell body proper and then leaves it as a distinct free flagellum ($13.6 \mu\text{m}$).

Type III (Flat, leaf-like form) [Fig. 1c & Plate I Fig. 3] :

In peripheral circulation and in kidney smears this form was found in abundance. Its anterior end is narrow and pointed, posterior end being sometimes, abruptly pointed or more or less rounded. The body, when examined in living condition, seems to be partially twisted about its longitudinal axis. The body is leaf-like, flattened and thinned out along the convex edge to form an undulating membrane. The cytoplasm densely granular which is more conspicuous in the posterior two-third of the body, stains deep blue and has striated myonemes (3-5 in number) along its longitudinal axis.

Oval or rounded nucleus measuring $2.4 \times 1.6 \mu\text{m}$ is situated on the posterior part of the body ($NI=0.3$). It stains purple red with Giemsa and seems to be karyosomatic. Kinetoplast, staining deep blue black with Leishman, very small ($0.8 \times 0.8 \mu\text{m}$), spherical or rod-like and surrounded by a halo, is situated at the extreme posterior end of the body ($KI=2.5$).

Flagellum, emerging from the kinetoplast and having 4-6 deep attachment with the cell body proper in the form of conspicuous undulating membrane, leaves the body as a distinct free flagellum ($24.0 \mu\text{m}$).

Type IV (Larger compact form) [Fig. 1d & Plate I Fig.4] :

This form is comparatively broader and stouter with broader posterior end. The body is typically twisted and measures $31.52 \mu\text{m}$ (PA) in length

and $7.78 \mu\text{m}$ in width. Cytoplasm, staining deep blue with Leishman and Wright's stains, is densely granular, more dense in the posterior sector and the longitudinally striated periplast is very conspicuous.

Spherical or oval nucleus with a prominent karyosome measures $2.2 \times 1.7 \mu\text{m}$ and is situated in the middle of the body. It stains purple red with Leishman. The kinetoplast measuring $0.8 \times 0.5 \mu\text{m}$, rounded dot-like or rod-shaped, is situated very close to the nucleus and stains deep blue. The flagellum, as usual, originating from the kinetoplast borders many conspicuous folds of the undulating membrane before leaving the cell body as a free flagellum, measures $6.3 \mu\text{m}$ in average.

Morphological variability in the trypomastigote stage of T rotatorium obtained from different Indian anuran hosts :

During the present investigation on anuran haematozoa, the authors have encountered *T rotatorium* from a number of heterologous species viz., *Rana tigrina*, *R. limnocharis*, *R. cyanophlyctis*, *Bufo melanostictus*, *B. stomaticus*, *Rhacophorus maculatus* and *R. malabaricus* with some new host records from Indian subcontinent. The variation in morphometric measurements of the typical trypomastigote obtained from the above mentioned seven anuran hosts has been enumerated in tables 4 and 5.

Divisional and developmental stages of T rotatorium in anuran hosts :

Extensive search and repeated examination through seasons in different anuran hosts revealed some divisional stages of *Trypanosoma rotatorium* in the peripheral blood, in the liver and kidney smears and also in the bone-marrow smears.

During the rainy season large leaf-like trypanosomes withdrew their flagella, became rounded and then division was seen to start with longitudinal binary-fission. In *R. tigrina*, a few amastigote and epimastigote forms were detected in the blood and bone-marrow smears on the advent of winter. In *Rhacophorus maculatus*, a good number of rounded amastigote forms (Fig. 4f, g) measuring $9.5 \times 8.0 \mu\text{m}$ were detected in the liver and kidney smears. Amastigote forms show lightly granulated bluish cytoplasm with a round chromatin dot and oval reddish nucleus; the nucleus measures $2.5 \times 1.5 \mu\text{m}$. In many instances the kinetoplast and nuclei underwent division while the cytoplasm of the amastigote and spheromastigote forms remained intact.

Some epimastigote (Figs. 4h & Plate II Fig. 7) were also detected from the liver smears of the same host *Rhacophorus maculatus*. These epimastigote forms are elongated measuring $28 \mu\text{m}$ in length (excluding free flagellum and $8.0 \mu\text{m}$ in width with tapering posterior end. The flagellum is $29 \mu\text{m}$ in

length and somewhat greater than the body (PA). The nucleus is broad, oval; stained purple with Leishman and situated towards the posterior end. It measures $5.0 \times 4.1 \mu\text{m}$. The dot-shaped kinetoplast is just behind the nucleus from which the flagellum emerges. The flagellum traverses the body and producing 3 small undulations at the anterior end and then leaves the body as a long free flagellum. The cytoplasm of the body is lightly granulated and stained light blue with Leishman and Giemsa stains.

In *Rhacophorus malabaricus* some divisional stages of the trypomastigote forms were observed. Some forms were found to have two nuclei and two kinetoplasts (Fig. 4a) and some with one nucleus and two kinetoplasts. But

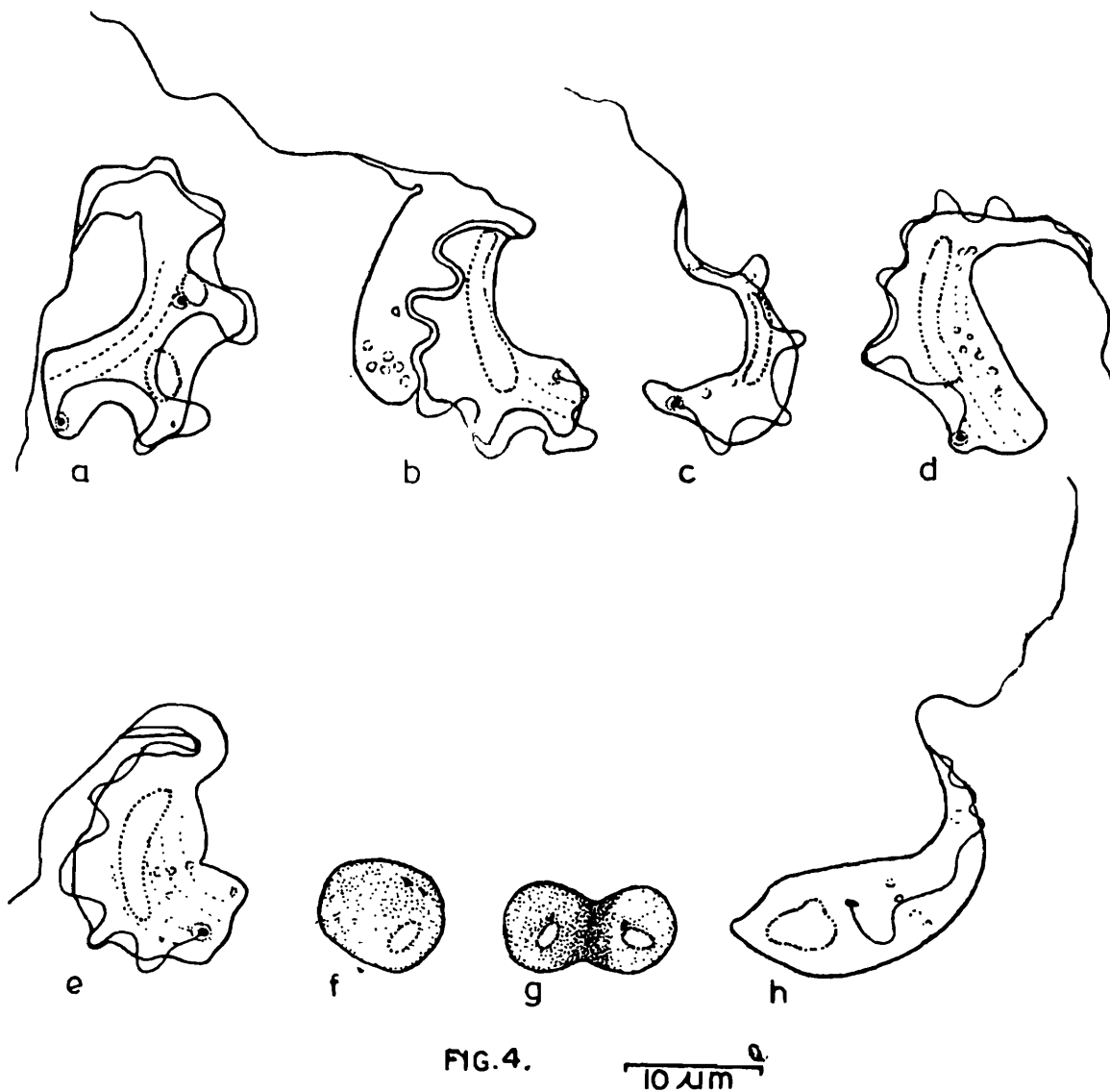


FIG. 4. Camera lucida drawings of *Trypanosoma rotatorium* from two anuran (Tree frogs) hosts. a & b, the trypomastigote stages in *Rhacophorus malabaricus*; c—h, Different stages of *T. rotatorium* in *Rhacophorus maculatus*; c, the slender form; d & e, the flat leaf-like trypomastigote form; f, the amastigote form; g, the dividing amastigote form with 2 kinetoplasts and 2 nuclei; h, the epimastigote stage.

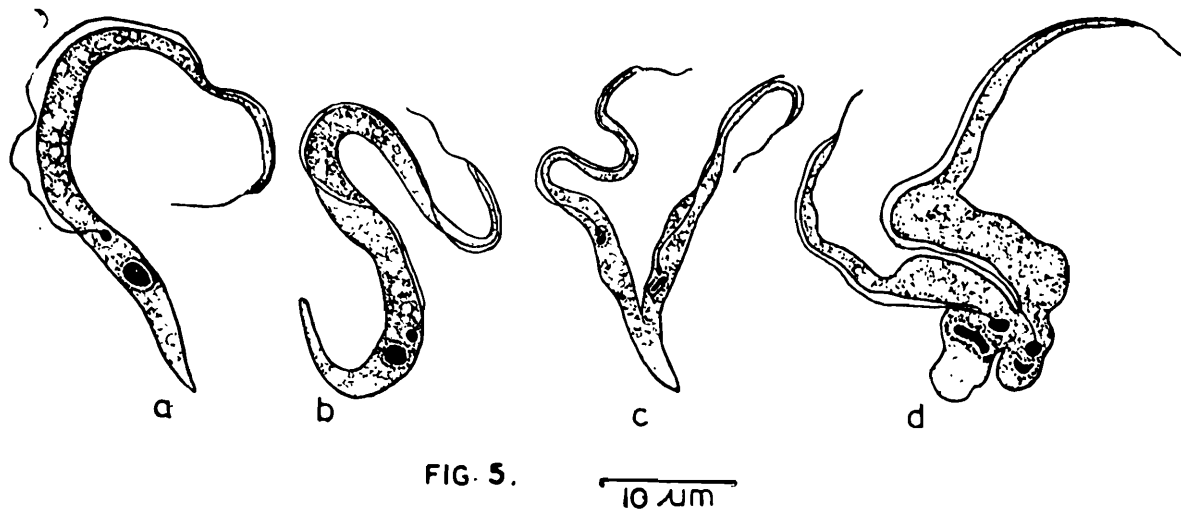


Fig. 5. Camera lucida drawings of the developmental stages of *Trypanosoma rotatorium* in the leech vector *Helobdella nociva*. a & b, the long slender epimastigote forms ; c, the dividing epimastigote form, showing equal longitudinal binary fission ; d, unequal division of long slender epimastigote form.

which one divides earlier is not clear and it seems that kinetoplast divides earlier than the nucleus.

In *Rana limnocharis* a typical dumbbell shaped dividing amastigote form (Fig. 2b & Plate II Fig. 8) has been detected in the peripheral blood where 4 nuclei and 4 kinetoplasts were encountered, two nuclei and two kinetoplasts in each half of dumbbell. It measures $20 \times 12.2 \mu\text{m}$. The stage represents an equal binary fission of the parasite which ultimately gives rise to 4 smaller rounded amastigote forms followed by a second division. Dividing trypomastigote forms with 2 nuclei and 2 kinetoplasts were also detected in the peripheral blood (Fig. 2c) of the same host.

Transmission of T rotatorium from frog to leech :

Trypanosoma rotatorium was experimentally transmitted through a laboratory reared Rhyncobdellid leech vector *Helobdella nociva*. The developmental stages inside crop and gastric caeca were studied and categorized as follows :

A) Epimastigote B) Spheromastigote C) Amastigote and D) Metacyclic forms (Fig. 5a-d, 6a-q and Plate III Figs. 1-8). Beside these some transitional forms were also encountered. Epimastigote forms are further subdivided into three categories *viz.*, 1) long slender epimastigote (Figs. 5a-d & 6a) 2) short slender epimastigote (Figs. 6b-d) and 3) stumpy short-membraned form (Figs. 6e-i & Plate III Figs. 7, 8) according to their shape and size of the body (Table 2).

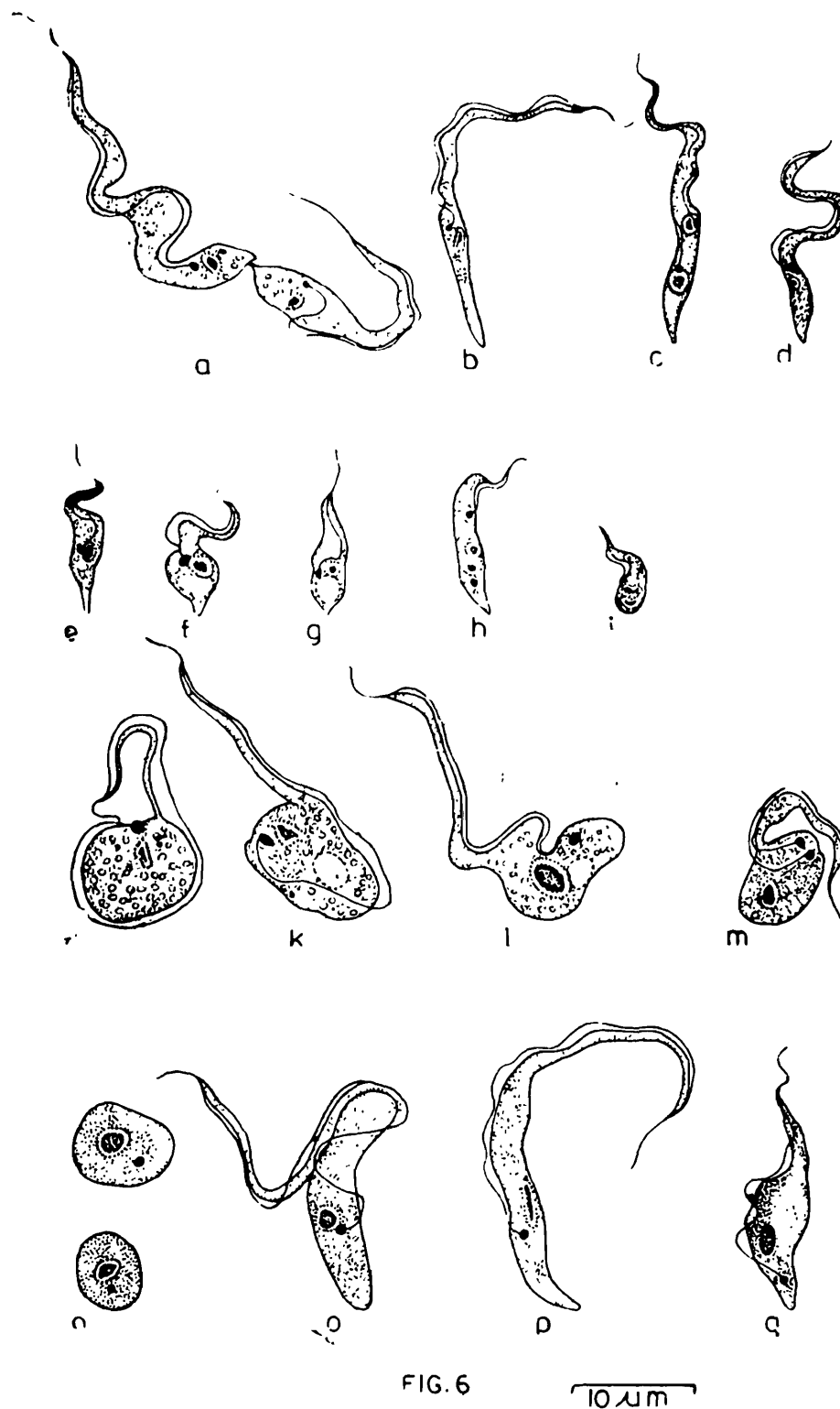


Fig. 6. Camera lucida drawings of the developmental stages of *Trypanosoma rotatorium* in the leech vector *Helobdella nociva*. a, the two newly formed epimastigote stages just after complete separation; b & d, The short slender epimastigote forms; c, dividing epimastigote stage; e-i, the stumpy short-membraned forms; j & l, the spheromastigote forms; m, the dividing spheromastigote form; n, the amastigote stages; o & p, the transitional metacyclic forms; q, the trypanosomal form just after inoculation by the leech.

The noted morphological changes were involved during the transition of the vertebrate forms to invertebrate forms in the leech vector. The flagellates display marked slendering of the body (epimastigote and metacyclic), thickening of the axoneme, increased refractibility of the cytoplasm, and disappearance of the metachromatic granules and myonemes. Furthermore, the serpentine movement of the body came to a halt but the wriggling motion on its longitudinal axis was retained. The relative number of developmental forms of *T. rotatorium* in leeches of different day of infection is enumerated in the table 3.

Transmission of T. rotatorium from leech to frog :

The experimental transmission of *T. rotatorium* to clean frogs was accomplished by exposing the frogs to infected leeches. Subcutaneous inoculations of the proboscis content were made. The blood of the frogs after inoculation and leech-feeding was examined and trypanosomal form of Type I and Type II were encountered after the 6th day of inoculation.

Remarks : During the first two decades of the present century a substantial volume of work on the anuran trypanosomes was published. An extensive review of the literature revealed that, although anuran trypanosomes were discovered a century ago (Gluge, 1842) and the present generic name *Trypanosoma* was given a year later (Gruby, 1843), their biology remained thoroughly studied.

Trypanosoma rotatorium (Mayer, 1843) is highly pleomorphic haemoflagellate having a wide host range. Various workers reported on it from different corners of the globe with many new host records (aprox. 70).

The present paper embraces the detail study of the morphology and biology of *T. rotatorium* along with three new host records from Indian sub-region (Ray, 1979 a, b, Ray 1980, Ray and Choudhury, 1981). *T. rotatorium* is being recorded for the first time from the family Rhacophoridae and *Helobdella nociva*, a Rhyncobdellid leech, has been experimentally demonstrated to be a possible vector for this anuran trypanosome (Ray and Choudhury, 1981).

On analysing the morphometric parameters and body ratios of *T. rotatorium* (Tables 4 & 5) from seven host species (*Rana tigrina*, *R. limncharis*, *R. cyanophlyctis*, *Bufo melanostictus*, *B. stomaticus*, *Rhacophorus maculatus* and *R. malabaricus*), it has been revealed that this trypanosome has retained more or less the same range of morphometric measurements with slight variations which are less significant. These variations are due to

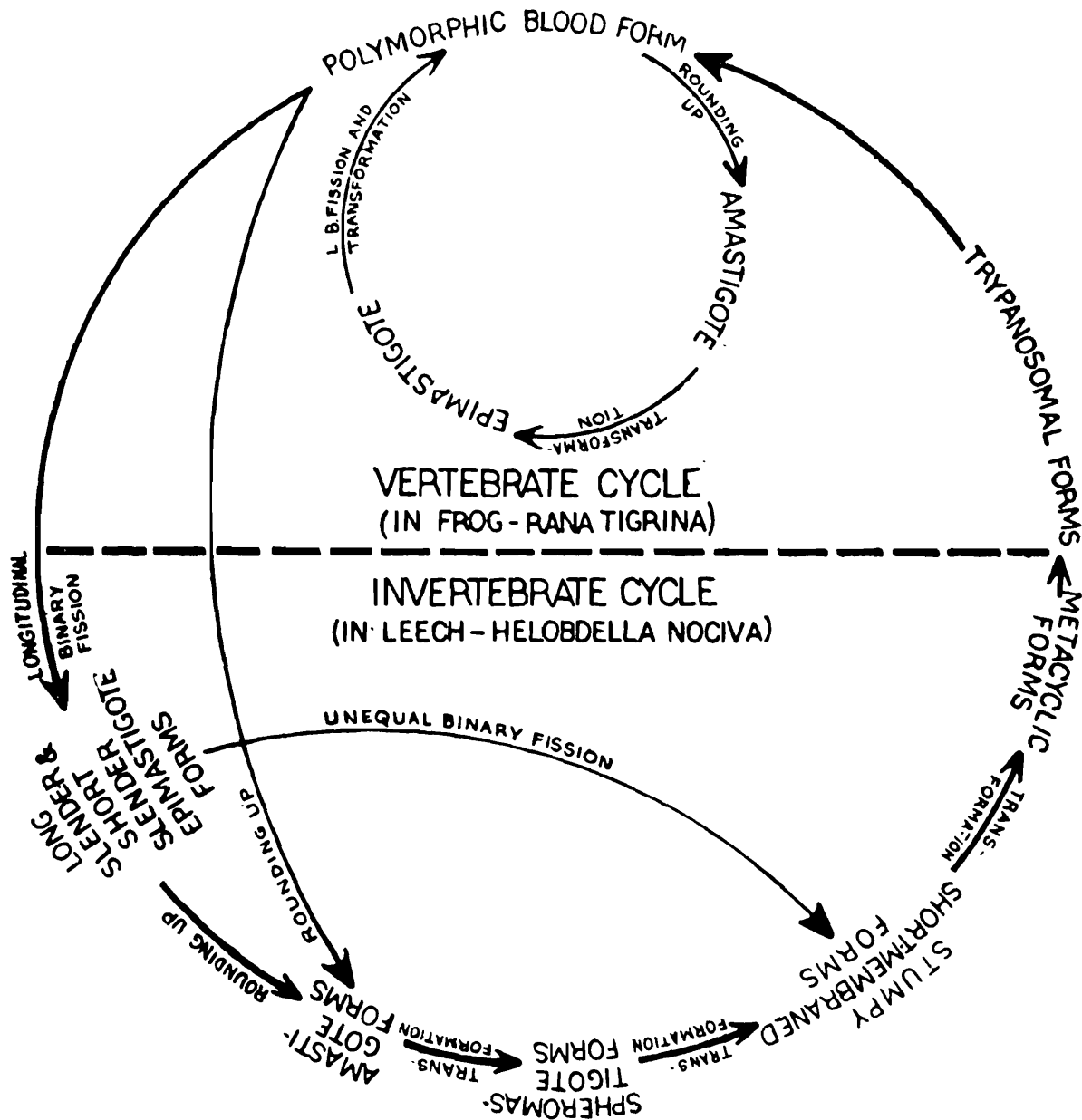
its different host interaction and ecologic variability. In *Bufo stomaticus* the trypanosome became more broader and longer, where the width of the body is greater than the one third of the body length. In *R. cyanophlyctis* and *Rhacophorus malabaricus* the free flagellum of the haemoflagellate is very much longer and it is about three-fourth of the body length. In *R. limnocharis*, the free flagellum is half the body length. The trypanosomes encountered from *R. tigrina* are smaller in width associated with short free flagellum and the ratio of PK/PA (0.21) is greater in comparison to its fellow members encountered from other hosts. From the kinetoplastic (KI) and nuclear index (NI), it has been revealed that the nucleus of the *T. rotatorium* was always situated in the posterior part of the body and the kinetoplast was proximated to the posterior end than to the nucleus except in *R. tigrina* where it was very close to the nucleus.

Relatively little evidence is in hand during the years of research on anuran trypanosomes on the type or types of reproduction occurring in the vertebrate hosts. Many authors who studied these haemoflagellates have found no detectable signs of reproduction either specifically in the peripheral blood (Franca, 1907 a, b, 1908, 1915 ; Nöller, 1913 a ; Kudo, 1922 ; Macfie, 1914) or in the frog as a whole (Seed, 1970). However, several authors have reported various types of divisions in anuran trypanosomes, e. g., binary fission (Diamond, 1958 ; Fantham *et al.*, 1942) and multiple fission of rounded amastigote stages (Buttner, 1966, Franca 1907a, Lehman, 1959).

In the present investigation the authors have noted binary fission in all the three stages of epimastigote, amastigote and trypomastigote of *T. rotatorium* in the vertebrate hosts *Rana limnocharis* and *Rhacophorus maculatus*. Multiple fission of the amastigote stage has not been encountered. Differing reports exist on the site of reproduction of *T. rotatorium*. Tanabe (1931) reported it from bone-marrow, Ivanic (1936) from kidney and Fantham *et al.*, (1942) from peripheral blood. This is being endorsed by the present authors who encountered the reproductive stages in liver and heart smears. The bone-marrow and kidney exhibited predominant divisional stages of trypomastigote and amastigote forms of *T. rotatorium*.

The account of *T. rotatorium* communicated in the present monograph well agrees with the data of Franca and Athias (1907) and Mathis and Léger (1911 c, d). But the length of the nucleus in Indian species is smaller in comparison to the foreign species. Fantham *et al.*, (1942) described the same parasite from *Rana catesbeiana* of Eastern Canada. They mentioned that the measurements of this trypanosomes were variable due to its polymorphic nature (free part of the flagellum was very short, in fact, there might be

hardly any free flagellum or none). Their description and measurements well corroborate to some respect to the Indian species which is little smaller in size and always with a well marked free flagellum of considerable length.



SCHEMATIC REPRESENTATION OF THE LIFE-CYCLE OF *T. ROTATORIUM*
FIG A

Relatively few intermediate hosts for the trypanosomes of cold-blooded vertebrates are known. A perusal of the literature revealed that Billet (1904) was the first worker who successfully infected *Helobdella algira* with *T. rotatorium* and noted its reproduction in the invertebrate host's gut. Franca (1907 a, b, 1908), Nöller (1912b) and Nigrelli (1944) studied the development of this parasite in *Hemiclepsis marginata*, *Placobdella parasitica* and *Macrob-della* sp. respectively. Brumpt (1906 a) and Barrow (1953) did some work

on the development of Newt trypanosomes in leech vector. Buttner and Bourcart (1955) and Diamond (1958) described the invertebrate part of *Trypanosoma inopinatum* in *Helobdella algira* and *Trypanosoma pipientis* in *Placobdella phalera* respectively.

The present authors studied the various developmental stages of *T rotatorium* in experimentally infected leech, *Helobdella nociva* Harding. Clean Juveniles of *H. nociva* were fed to infected *R. tigrina*. Developmental stages inside the leech-vector have been categorized and correlated. A possible scheme of life-cycle of *T rotatorium* is depicted in Fig. II. The developmental cycle of *T rotatorium* in the leech vector seems to be similar to that of trypanosomes of fishes and other amphibians in that, epimastigotes were present initially and these later changed into transitional and finally to metacyclic forms through a series of differentiation stages. Multiplication by binary fission in the crop and gastric caeca of the leech is similar to that described for other trypanosomes.

The significant part of the present study is that the authors have encountered 3 distinct types of epimastigote forms which are produced by the unequal division by longitudinal binary fission of the trypomastigotes and also the epimastigote forms. The authors intend to designate the rounded forms with short flagellum as spheromastigotes which undergo transformation like Diamond's (1958) type II b and ultimately develop into the stumpy short-membraned forms. Most of the workers reported the presence of metacyclic and large number of crithidial stages in the proboscis sheath of infected leech (Brumpt, 1906 b ; Nöller, 1913 b ; Robertson, 1907, 1909 b, 1912 ; Diamond, 1958) from which infection took place to the anuran hosts during the feeding. In the present investigation the authors have observed the presence of large number of metacyclic stages admixed with a few transitional and stumpy short-membraned forms in the proboscis sheath of the leech host *Helobdella nociva*. On feeding to new uninfected *R. tigrina* by the infected leech, *T rotatorium* was successfully established in the anuran host.

2. *Trypanosoma loricatum* (Mayer)

(Figs. 7a-d & Plate IV Figs. 1 & 2)

- 1843. *Paramaecium loricatum* Mayer, 'Spicilegium observationum anatomicarum de organo electrico in *Ralis anelectricis* et de Haematozois' Bonnae.
- 1843. *Paramaecium costatum* Mayer, *Ibid.*
- 1843. *Trypanosoma sanguinis* Gruby, *C. r. séal. Acad. Sci.*, 17 : 1134 ; Danilewsky, 1885, *Biol. Zbl.*, 5 : 529 ; 1889, La Parasitologie comparée du sang 1. Nouvelles recherches sur les parasites du sang des Oiseaux, Kharkoff, Partim.
- 1882. *Paramacioides costatus* Grassi, *Atti. Soc. Ital. Sc. Nat. Milano.*, 24 : 135.

1885. *Trypanomonas ranarum* Danilewsky, 1885, *Biol. Zbl.*, 5 : 529 ; 1889, La parasitologie comparée du sang 1. Nouvelles recherches sur les parasites du sang des Oiseaux, Kharkoff, Partim.
1885. *Trypanosoma ranarum* Danilewsky, *Ibid* ; 1889, *Ibid*.
1888. *Trypanosoma sanguinis ranarum* Shalshnikov, *Arkh. Vet. Nauk. S. Peterburg.*, 1 : 65, Partim.
1889. *Trypanomonades ranarum* Danilewsky, La parasitologie comparée du Sang. 1. Nouvelles recherches sur les parasites du Sang des Oiseaux, Kharkoff, Partim.
1889. *Trypanosoma costatum* Danilewsky, *Ibid*. Partim ; Franca and Athias, 1906, *Archs. Inst. Real. Bact.*, 1 : 127, Partim.
1889. *Trypanosoma costatum anarum* Danilewsky, La parasitologie comparée du sang. 1. Nouvelles recherches sur les parasites du Sang des Oiseaux, Kharkoff, Partim.
1906. *Trypanosoma loricatum* vel *costatum* Franca and Athias, *Arch. Inst. Real. Bact.*, 1 : 127, Partim ; Dutton *et al.*, 1907. *Ann. trop. Med. Parasit.*, 1 : 287.
1906. *Trypanosoma loricatum* Franca and Athias, *Arch. Inst. Real. Bact.*, 1 : 127 ; Pérez-Réyes, 1967, Doctoral thesis, Mexico ; Miyata, 1978 *Trop. Med.* (Nagasaki), 20 (1) : 51 ; Ray, 1979b Doctoral thesis, Calcutta University.
1906. *Trypanosoma rotatorium* Bouet, *Annls. Inst. Pasteur. Paris*, 20 : 564, Partim ; Hunter, 1908, *Hong Kong Rep. advis. Trop. Dis. Res. Fund* (1907) ; 108, Partim ; Labedeff, 1910, *Richard Hertwigs* (Munche), 1 : 397, Partim ; Mathis and Léger 1911 b, *C. r. Séanc. Soc. Biol.*, 70 : 956 ; 1911 c, *C. r. Séanc. Soc. Biol.*, 70 : 1008, Partim ; Noller, 1913 a, *Arch. Protistenk.*, 28 : 313 ; 1913 b, *Arch. Protistenk.*, 31 : 169, Partim ; Ogawa and Uegaki, 1927, *Arch. Protistenk.*, 57 : 14, Partim ; Tanabe, 1931, *Keijo J. Med. Japan*, 2 : 53, Partim ; Jacono, 1938, *J. Trop. Med. Hyg.*, 41 : 53, Partim ; Acanfora, 1939, *Arch. Ital. Sci. Med. Colon.*, 20 : 625 Partim.

Type-host : *Rana esculenta* (Linn.)

Other hosts : *Rana pipiens*, *R. guntheri*, *R. nigromaculata*, *R. galamensis*, *R. oxyrhynchus*, *R. mascaranensis*, *R. plancyi*, *R. tigrina*.

New Indian host : *Rana limnocharis* Wiegmann

Locality : Balitha and Bishnupur Bankura district, West Bengal, India.

Prevalence : Out of 307 examples of *R. limnocharis* examined, 55 (17.9%) were found to be positive.

Description : *Trypanosoma loricatum* is dimorphic and the authors have encountered two distinct varieties of trypomastigote, viz., Type I slender form and type II broad costate form.

Earlier reports on this species were concerned with the description of the trypomastigote designated by the writers as type II. An information regarding the morphology of the type I stage is absolutely wanting and even the biology of the species as a whole.

The morphology of the two trypomastigote forms are as follows : Type I (slender form) [Figs. 7a & Plate IV Fig. 1] :

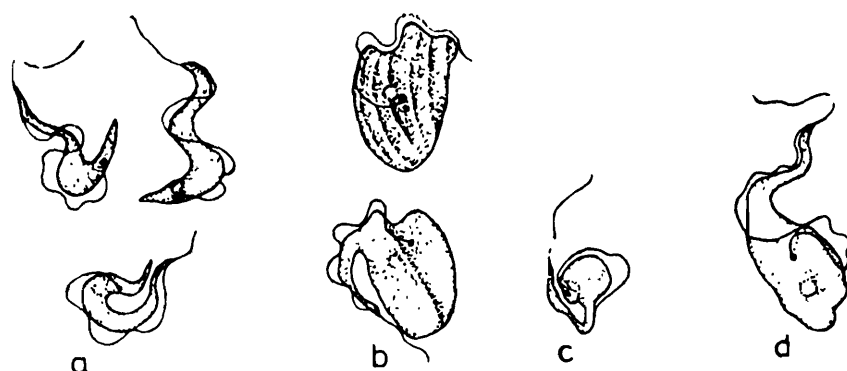


FIG. 7

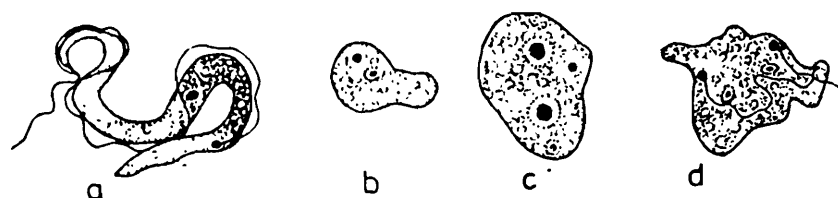
10 μ m

FIG. 8

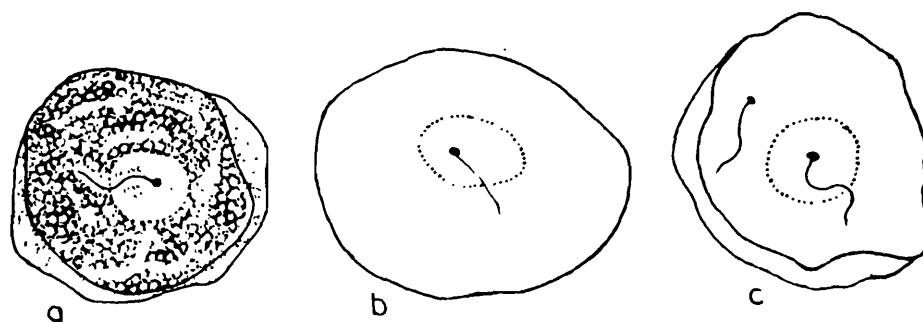
10 μ m

FIG. 9

10 μ m

Fig. 7. Camera lucida drawings of *Trypanosoma loricatum* in *Rana limnocharis*. a, Type I trypomastigote form; b, Type II trypomastigote form; c, the spheromastigote stage; d, the epimastigote stage.

Fig. 8. Camera lucida drawings of *Trypanosoma karyozeukton* in *Rana hexadactyla*. a, the trypomastigote stage; b, the amastigote form; c, the dividing amastigote form with 2 kinetoplasts and 2 nuclei; d, the dividing trypomastigote stage.

Fig. 9. Camera lucida drawings of *Trypanosoma chattoni* from two anuran hosts. a & b, *T. chattoni* in *Rana limnocharis*; c, Rounded dividing form of the same parasite in *Rana cyanophlyctis*.

These are elongated slender individuals, partially twisted about the longitudinal axis. It widens anteriorly from the kinetoplast and narrows posteriorly. The parasites measure $16.25 \mu\text{m}$ in length (excluding free flagellum) and $2.34 \mu\text{m}$ in width i.e. $1/7$ part of the body length ($\text{BW/PA} =$

0.14). Sometimes the posterior end is drawn out into a slender thread like structure.

Cytoplasm is homogeneous, finely granular and without any striation or costate lines and always stained light blue with Leishman and Giemsa stains. Nucleus is rounded to oval measuring $1.01 \mu\text{m}$ in length and $0.5 \mu\text{m}$ in width ; stained dark red with Romanowsky type of stains. It is located in the posterior end of the body near the kinetoplast (PN/PA=0.26).

Kinetoplast is a small rod-like structure measuring $0.41 \mu\text{m} \times 0.42 \mu\text{m}$ and stained deep blue with Leishman. It is situated on the posterior end of the body very close the nucleus (KI=4.79).

The flagellum arises from the posterior part of the kinetoplast and traverses the body in the form of an undulating membrane with 3-5 short folds (width= $1.08 \mu\text{m}$) and ultimately leaves the body as a distinct free flagellum measuring $5.85 \mu\text{m}$ in length which is one third of the body length (FF/PA =0.35).

Type II (Broad form) [Fig. 7b & Plate IV Fig. 2] :

These forms are found abundantly in the peripheral circulation of the frogs. These are thick and broad with rounded or oval posterior and attenuated anterior end. Sometimes twisted on the longitudinal axis. They measure $20.76 \mu\text{m}$ in length (excluding free flagellum) and $6.70 \mu\text{m}$ in width, which is approximately one-third of the body length (BW/PA=0.31).

Cytoplasm is thick, densely granular, stained deep blue. The subpellicular surface is distinctly costate. Costae are 7-9 in number. The costate condition of the body surface becomes apparent by the degree of flattening to which the parasite is subjected. Thus in the thicker portions of the surface of the parasite distinct costae are visible, where as in the thinner portion, the surface seems often to be completely smooth.

Nucleus is rounded to oval in structure measuring $1.4 \times 1.04 \mu\text{m}$; stained deep red with Leishman. It is situated in the middle-third of the body near the kinetoplast (PN/PA=0.37).

Kinetoplast is small, dot-like structure, measuring $0.48 \mu\text{m}$ in diameter, located submarginally near the nucleus on the posterior third of the body (KI=6.0).

The flagellum arises from the basal granule of the kinetoplast and traverses the body forming an undulating membrane (width= $1.2 \mu\text{m}$) with

3—4 short folds and finally leaves the body as a very short free flagellum measuring $4.96 \mu\text{m}$ in length which is more than one-fifth of the body length (FF/PA=0-23).

A comparison of the morphometric measurements of the two trypomastigote forms are presented in the Graph-I and II (also vide Tables 6 & 7).

Developmental stages inside the vertebrate hosts :

Extensive study and repeated examination revealed some developmental stages of *T. loricatum* in the peripheral blood and in the imprint smears of liver and kidney.

The trypanosomes withdraw the undulation and become spherical or rounded to form spheromastigote stage (Fig. 7c). These spheromastigote forms having a short undulation and flagellum, divide by means of simple binary fission. They measure $11.25 \mu\text{m}$ in length and $4.0 \mu\text{m}$ in width. The free flagellum measures $7.75 \mu\text{m}$ in length.

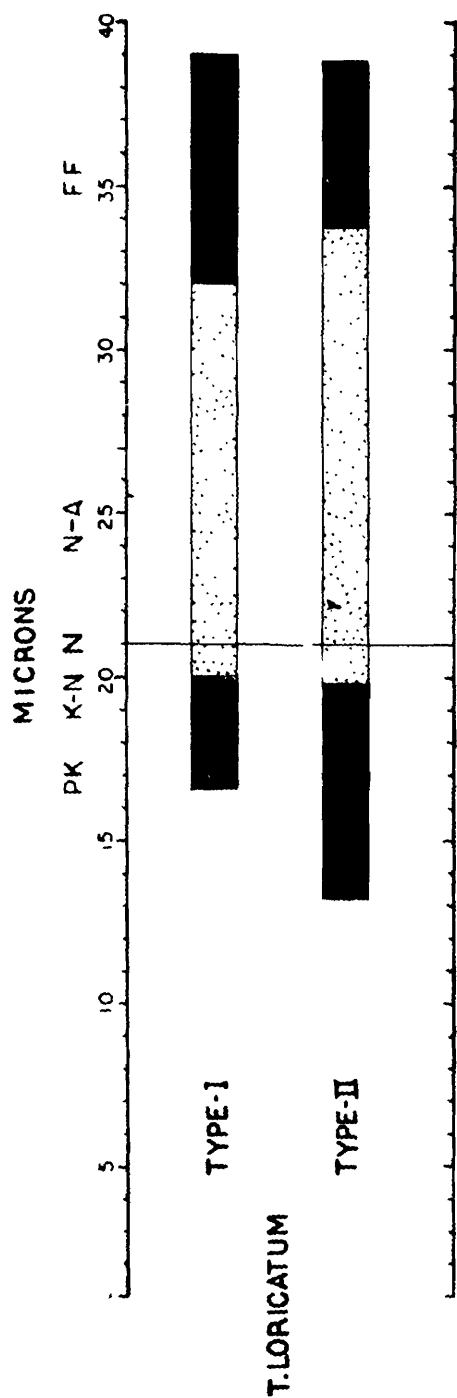
Some epimastigote stages were also encountered in the kidney smears (Fig. 7d). They measure $24.0 \mu\text{m}$ in length and $5.5 \mu\text{m}$ in width. The dot-like kinetoplast is situated anterior to the oval nucleus. The free flagellum is $10 \mu\text{m}$ in length. Cytoplasm is lightly granular and non-vacuolated, stained light blue with Leishman and Giemsa stain. No amastigote stages were recorded so far.

Exposure to possible leech vectors :

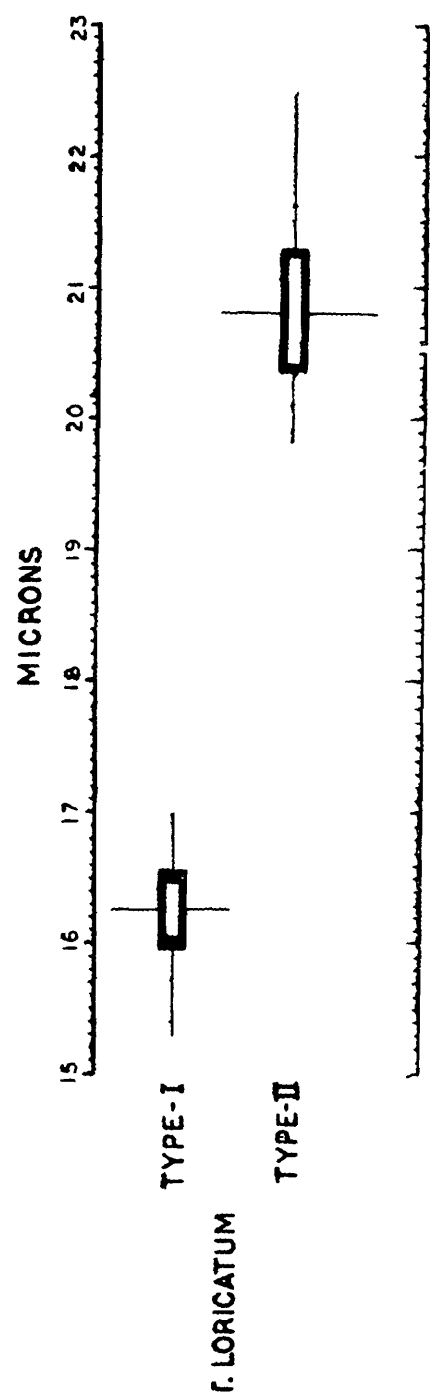
T. loricatum was experimentally tried to infect the leeches like *Helobdella nociva* and *Hemiclepsis marginata*. But development did not take place inside those leeches.

Remarks : Mayer (1843) was the first to observe this parasite in the blood of European frog, *Rana esculenta* and named *Paramaecium loricatum* which was presumed to have an appearance like a ciliate, *Paramaecium*. Mayer, as mentioned above, described the costate trypanosomes under the name of *P. loricatum*. However, when he labelled the sketches of this organism he employed the name *Paramaecium costatum*. This has led to considerable confusion in nomenclature. Later on, Diamond (1958) synonymized the *P. costatum* with *P. loricatum*. There is no doubt to day that Mayer observed a trypanosome which superficially resembled a ciliate and which had been reported repeatedly from *Rana esculenta* and other anurans. In the review of taxonomic literature on anuran trypanosomes Diamond (1958) clearly stated that, a number of species (vide list of

synonymies) are nothing but *T. loricatum* and accordingly he also synonymized them with *T. loricatum*. Recently Pérez-Réyes (1967) reported this haemoflagellate from *Rana pipiens* of Mexico.



GRAPH-I
Comparative overall morphometric measurements of Type I and Type II peripheral blood forms of *Trypanosoma loricatum*. PK—Posterior end to the kinetoplast; KN—Kinetoplast to the centre of the nucleus; N—Centre of the nucleus; NA—Centre of the nucleus to the anterior end; FF—Free flagellum.



GRAPH-II
Comparison of variation in body (PA) length of Type I and Type II peripheral blood forms of *T. loricatum* as presented in Table 6 (Method of Dice Squares). A vertical line indicates the mean and a horizontal line, the range. On either side of the mean, black rectangles represent the standard deviation and white rectangle twice the error of the mean.

This parasite has been recorded by the present authors from a good number of Indian Paddy-field frogs, *Rana limnocharis*. The authors propose to designate the parasite *T. loricatum* as being dimorphic displaying two distinct forms with slender and broad oval costate body. In almost all the host individuals, these two forms were always prevalent.

In the present communication detail morphometric measurements and the relation of the different body parts (Tables 6 & 7) with one another for *T. loricatum* have been made for the first time from India. Besides, the developmental stages inside the vertebrate host viz., spheromastigote, epimastigote stages have been added to its life-cycle. Comparison of overall morphometric measurements and variation in body length of type I and type II forms of *T. loricatum* are represented in Graphs I and II respectively.

T. loricatum has got certain resemblances with *T. ranarum* in general appearance, in the presence of costae and position of kinetoplast. But it differs markedly in having thick body with broad rounded posterior end, dot-like kinetoplast, short flagellum and other morphological parameters (Table 6).

The morphometric data given by Franca and Athias (1906) and Mathis and Léger (1911a) indicate that the parasite described by them were larger than the present one. Although they were larger in size yet the ratio BW/PA corroborates more or less with the present parasite.

The authors contend that these forms belong to same and one species, and represent merely geographic and ecologic (including heterologous host interactions) variability.

3. *Trypanosoma karyozeukton* Dutton and Todd

(Figs. 8a-d & Plate IV Fig. 3)

1903. *Trypanosoma karyozeukton* Dutton and Todd, *Mem. Lpool. Sch. Trop. Med.*, 11 : 1 ; Rodhain, 1907, *Zbl. Bakt.*, 45 : 129 ; Martin *et. al.*, 1909, *Bull. Soc. Path. exot.*, 2 : 209 ; Franca, 1925, *Annl. Parasit. hum. comp.*, 3 : 225 ; Schwetz, 1930, *Annl. Parasit. hum. comp.*, 8 : 122 ; 1938, *Annl. Parasit. hum. comp.*, 16 : 242, 1944, *Annl. Soc. belge Med. trop.*, 24 : 263, Partim ; Lauter, 1960, Doctoral Thesis, Louisiana State Univ., U.S.A. ; Ray, 1980, *Indian J. Parasitology*, 3 (suppl.) : 87.
1907. *Trypanosoma loricatum* Dutton, Todd and Tobey, *Ann. Trop. Med. Parasit.*, 1 : 287, Partim.
1907. *Trypanosoma loricatum vel costatum* Dutton, Todd and Tobey, *Ibid*, Partim.

Type host : *Rana* spp. (?)

New Indian host : *Rana hexadactyla* Lesson

Locality : Salikona, Hooghly district, West Bengal, India.

Prevalence : Out of 180 frogs examined, 25 (13.8%) were positive for this trypanosome.

Description : The following description is based on the trypanosome

collected from an Indian green frog, *Rana hexadactyla*. The trypanosomes were found in the peripheral blood of the frogs.

The body is elongated and coiled in the form of a helix (Fig. 8a & Plate IV Fig. 3) measuring $44.64\mu\text{m}$ ($43\text{-}47\mu\text{m}$) excluding free flagellum. Both the extremities tapered gradually to points. Body is very thin and vermiform; surface is not smooth; width $2.64\mu\text{m}$ ($2.5\mu\text{m} - 3.0\mu\text{m}$).

Cytoplasm is granular in the pre-nuclear region; stained deep blue with Leishman and Giemsa stains. The post-nuclear region has fine granulation which stained light blue. Chains of granules in between the nucleus and kinetoplast are not well marked. Costae are conspicuous in dark stained specimens.

The kinetoplast is small, rounded or elliptical, stained deep blue. It is situated on the posterior part of the body in a position usually half the distance from the posterior extremity to nucleus ($KI=1.89$). Sometimes a halo surrounds the kinetoplast.

The nucleus is round to oval, measuring $2.34 \times 1.24\mu\text{m}$; stained red with Leishman. It is situated near the junction of the middle and posterior-third of the body and parallel to the body axis ($NI=0.79$).

The flagellum arises from the posterior part of the kinetoplast and traverses the whole body in the form of an undulating membrane ($1.38\mu\text{m}$) with 4-7 narrow folds and lastly emerges from the body as a free flagellum. It stained well with Leishman and measure $9.7\mu\text{m}$ ($9.5\text{-}10.0\mu\text{m}$) which is less than half the length of the body ($FF/PA = 0.21$).

Divisional stages (Figs 8 b, c, d) : Some divisional stages were observed both in the peripheral blood and kidney smears. Some rounded or dumb-bell shaped amastigote forms with 1 nucleus and 1 kinetoplast and sometimes 2 nuclei and 2 kinetoplasts were visible which indicated the dividing stages of the trypanosomes. Amastigote with IN, IK (Fig. 8b) measures $8.5 \times 4.0\mu\text{m}$ and $26\mu\text{m}^2$ in average area. The amastigote forms with double nuclei and kinetoplasts (Fig. 8c) are somewhat larger, measuring $12 \times 8.5\mu\text{m}$ with an average area of $62.5\mu\text{m}^2$. Some forms with two flagella (Fig. 8d) were also noted in the kidney smears.

Vector is unknown and no life-cycle stage has been recorded so far.

Remarks : *Trypanosoma karyozeukton* was first described by Dutton and Todd 1903, from certain frogs of Sanegambia. He and his associates also recorded the same species from *Bufo regularis*, *Rana mascarensis*, *R. occipitalis* and *R. oxyrhynchus*, in 1907. After that a number of workers

viz., Rodhain, 1907 ; Martin *et al.*, 1909 ; Franca, 1925 ; Schewetz, 1930 1944 and Lauter, 1960 recorded this parasite from the frogs and toads of Angola and Congo.

Dutton *et al.* (1907) described this parasite as polymorphic *viz.*, small, medium and large forms having three distinct morphometric parameters.

T. karyozeukton recorded by the authors from India is well corroborated with that of Dutton *et al.* (1907) and in every respect (Table 10). But only one form was encountered in *Rana hexadactyla* instead of three forms.

Moreover, some divisional stages were observed in the peripheral blood and kidney smears. Morphometric ratios of different body parts were recorded (Tables 8 & 9) for the first time. The species is also being recorded for the first time from Indian subregion along with the new host record and geography.

4. *Trypanosoma chattoni* Mathis and Léger

(Figs. 9a-c, 10a-i & Plate IV Figs. 4-7, Plate V Figs. 1-5)

1907. *Trypanosoma rotarium* Franca and Athias, *Archs. Inst. Real. Bact.*, 1 : 289, Partim ; Ivanic, 1936, *Cellulo*, 44 : 349, Partim ; Fantham *et al.*, 1942 *Parasitology*, 34 : 199, Partim ; Nigrelli, 1945, *Zoologica*, 30 (5) : 47, Partim ; Vucetich and Giacobbe, 1949, *An Inst. Med. Reg. Tucuman.*, 2 : 225, Partim.
1907. *Trypanosoma loricatum* Dutton, Todd and Tobey, *Ann. trop. Med. Parasit.*, 1 : 287 Partim.
1907. *Trypanosoma loricatum vel costatum* Dutton, Todd and Tobey, *Ibid.*, Partim.
1908. *Trypanosoma hylae* Franca, *Aychs. R. Inst. Bact.*, 2 : 271, Partim.
1911. *Trypanosoma chattoni* Mathis and Léger, *C. r. Séanc. Soc. Biol*, 70 : 956 ; Diamond, 1958, Doctoral Thesis Minnesota Univ. U.S.A. ; Miyata, 1978, *Trop. Med. (Nagasaki)*, 20 (1) : 51 ; Ray, 1979 b, Ph. D. Thesis, Cal. Univ., 1980, *Indian J. Parasitology*, 3 (Suppl.) : 87.
1936. *Trypanosoma cellestinoi* Brumpt, "Préces de Parasitologie" Masson et cie, Paris, 5th ed. pp. 290, 300.

Type-host : *Bufo melanostictus* Schneider.

Present Indian record : *Bufo melanostictus*, *Bufo stomaticus*, *Rana limnocharis*, *R. tigrina*, *Rhacophorus maculatus*, *R. malabaricus*, *Microhyla ornata*.

Locality : Volpoi, Mollem and Bondla, Goa, India.

Prevalence : 30 out of 343 (9.62%) *Bufo melanostictus*, 11 out of 73 (15.06%) *B. stomaticus*, 41 out of 307 (13.3%) *Rana limnocharis*, 55 out of 450 (12.2%) *R. cyanophlyctis*, 47 out of 177 (26.5%) *Rhacophorus maculatus*,

2 out of 27 (7.4%) *R. malabaricus*, 5 out of 164 (3.04%) *Rana tigrina* and 3 out of 144 (2.08%) *Microhyla ornata* were found positive for *T. chattoni*.

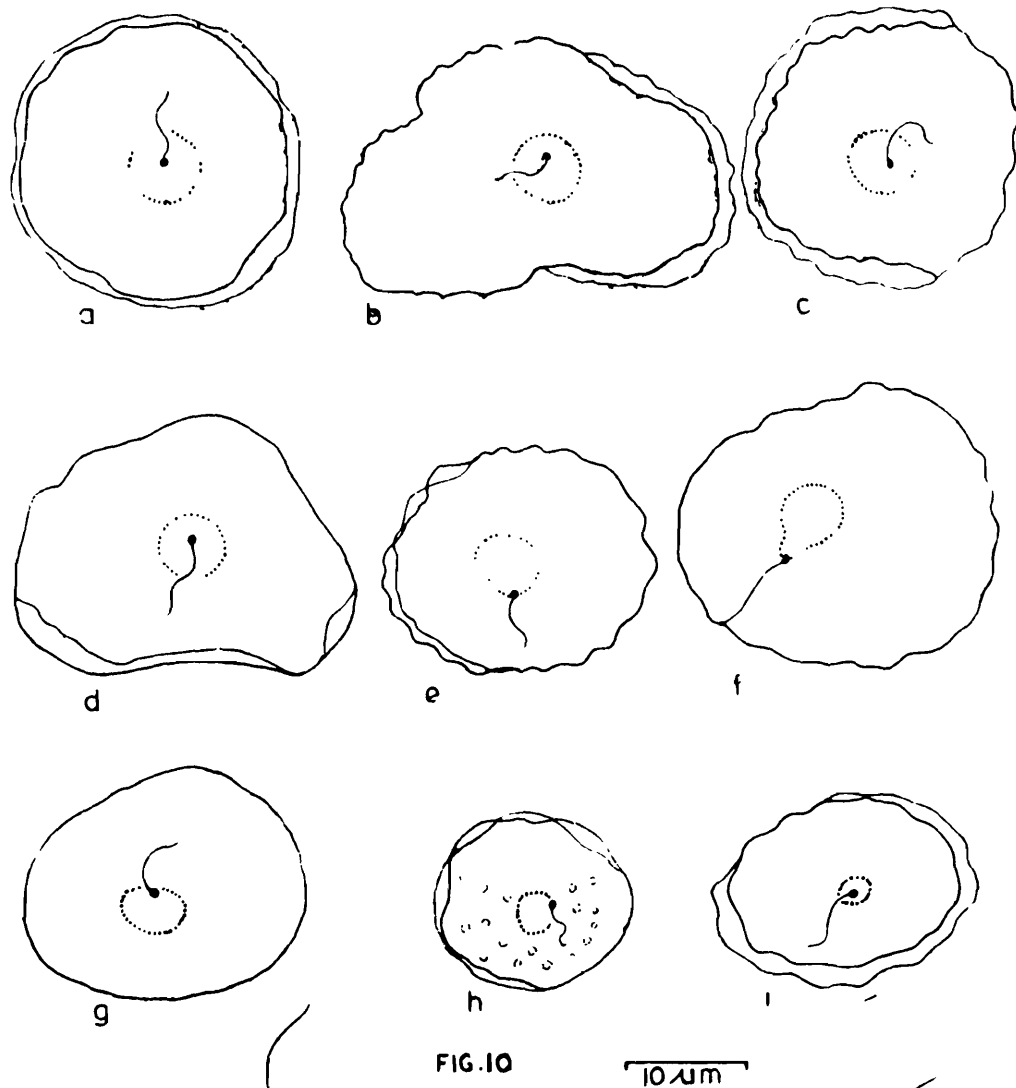


FIG. 10

10 μm

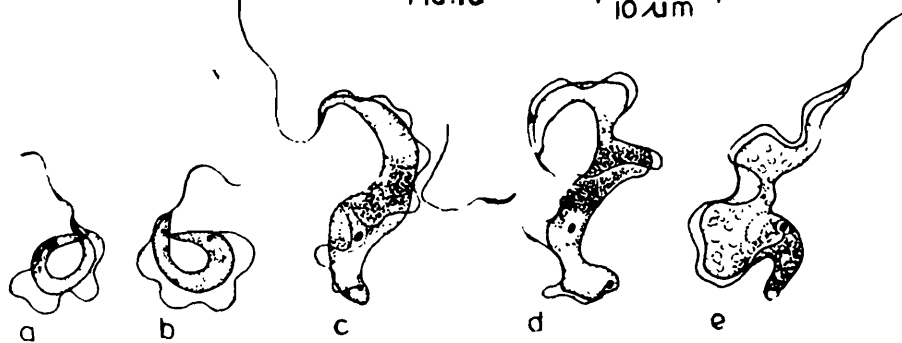


FIG. 11

10 μm

Fig. 10. Camera lucida drawings of *Trypanosoma chattoni* from six anuran hosts. a, *T. chattoni* in *Rana tigrina*; b & c, *T. chattoni* in *Bufo stomaticus*; d & e, *T. chattoni* in *Bufo melanostictus*; f, *T. chattoni* in *Microhyla ornata*; g, *T. chattoni* in *Rhacophorus malabaricus*; h & i, *T. chattoni* in *Rhacophorus maculatus*.

Fig. 11. Camera lucida drawings of three species of trypanosomes from three anuran hosts. a & b, *Trypanosoma taprobanica* sp. nov. in *Koloula pulchra taprobanica*; c & d, *Trypanosoma malabarica* sp. nov. in *Rana malabarica*; e, *Trypanosoma systema* sp. nov. in *Uperodon systema*.

Description : *Trypanosoma chattoni* has been recorded from a number of anuran hosts, *Bufo melanostictus*, *B. stomaticus*, *Rana tigrina*, *R. limncharis*, *R. cyanophlyctis*, *Rhacophorus maculatus*, *R. malabaricus* and *Microhyla ornata* from the same geographical region (Nova Goa, India) where the above mentioned host species inhabit. The following description is based on the species observed in *Bufo melanostictus* along with the morphometric variations of *T. chattoni* in other heterologous host species.

Observation on living specimens : Freshly drawn blood mixed with saline citrate solution (3 : 1) was observed under the microscope. The living organisms were typically spherical in shape with a smooth or sometimes irregular wrinkled surface ; the former was recognized as smooth form and latter as the rough form. Both the forms, at higher magnification appeared to be highly refractile bodies which could be readily dismissed as artifacts or abnormal host cells.

At the higher resolution they appeared as spherical protoplasmic bodies containing numerous moving refractile granules of varying size. A central refractile region, the nucleus, was also visible from which a short furrow originated in many species. These furrows contained short flagella. Almost all the individuals exhibited a beautiful whorling movement which could better be described as a discontinuous jerky rotation which proceeded in one direction for a time and then reversing completely in opposite direction.

Observation on stained specimens : In air-dried, Giemsa and Leishman stained blood films of *B. melanostictus*, a large number of rounded, spherical or sometimes irregular trophozoite bodies were observed (Fig. 9a). Some of them having smooth surface and others having irregular wrinkled surfaces. They measure $21.83 \mu\text{m}$ in diameter and $373.64 \mu\text{m}^2$ in area. The cytoplasm stained deep blue in colour and always contained innumerable metachromatic granules and fine vacuoles.

The more or less centrally placed nucleus is characteristically circular measuring $6.62 \mu\text{m}$ in diameter and $29.26 \mu\text{m}^2$ in area and stained deep red with Leishman and Giemsa. A very small circular or elliptical kinetoplast, measuring $0.51 \mu\text{m} \times 0.45 \mu\text{m}$ was observed to lie very close to, in contact with or superimposed upon the surface of the nucleus.

The flagellum is very short, either straight or curved, measuring $4.59 \mu\text{m}$, emerging from a basal granule adjoining the kinetoplast. In some cases it has been observed to lie in clear narrow canal-like space which extends from the basal granule to a certain length of the cytoplasmic body.

Morphological variations of *T chattoni* in other anuran hosts : *Trypanosoma chattoni* has been recorded from a number of Anuran hosts belonging to the family Bufonidae, Ranidae, Rhacophoridae and Microhylidae. It bears more or less similar structure and configurations in the heterologous hosts of course with certain morphological variations as presented in Table 11.

Exposure to possible leech vector : They were tried with the leech *Helobdella nociva* but no developmental stages occurred inside the leech's gut.

Remarks : *Trypanosoma chattoni* was first recorded by Mathis and Léger (1911a) from the toad, *Bufo melanostictus* of Vietnam. Previously Franca and Athias (1907) described some rounded forms and divisional stages of trypanosome in *Hyla arborea* as *T rotatorium*. Those stages were probably *T chattoni* (vide Diamond, 1958). After that a similar situation happened when Ivanic (1936), Fantham *et al.* (1942), Nigrelli (1945), Vucetich and Giacobbe (1949) described the trypanosomes as *T rotatorium*. But Diamond (1958) synonymized them all, described by the earlier authors as *T chattoni* and he described the same parasite from *Rana pipiens* of Minnesota. Diamond (1958) experimentally transmitted this parasite to the laboratory reared clean frogs and was able to maintain the strain in vivo. But from his seven years of studies he came to the conclusion that, as the trypanoform stages had never been observed both in the vertebrate host and culture media so the generic status of *T chattoni* might be questioned.

The present authors recorded the parasite once again in a number of hosts from the same geographic area which revealed that *T chattoni* has multiple choice of its host like that of *T rotatorium*.

From the comparative morphometric parameters (Table 11) of *T chattoni* in different hosts, it can be stated that this parasite undergoes certain morphological variations due to heterologous host interactions and became larger in *Bufo stomaticus* and smaller in *Rhacophorus maculatus*.

The present study corroborates well with Diamond (1958) in all respects except that only binary fission and no multiple fission has been observed in any of the Indian hosts.

5. *Trypanosoma inopinatum* Sergent and Sergent

(Fig. 12)

1904. *Trypanosoma inopinatum* Sergent and Sergent, *C. r. Séanc Soc. Biol.*, 56 : 123 ; Franca and Athias, 1906, *Archs. Inst. Real. Bact.*, 1 : 127 ; Brumpt, 1910, *Précis de parasitologie*, Paris, p. 121 ; Buttner and Bourcart, 1955, *Annl. Parasit. hum. comp.*, 30 : 431, Partim.

1906. *Trypanosoma undulans* Franca and Athias, *Archs. Inst. Real. Bact.*, 1 : 127 ; Franca, 1911, *Archs R. Inst. Bact.*, 3 : 229 ; Franca, 1912, *Bull. Soc. Path. exot.*, 5 : 99.
1906. *Trypanosoma elegans* Franca and Athias, *Archs. Inst. Real. Bact.*, 1 : 127.
- *1908. *Trypanosoma hendersoni*, Patton, *Ann. Rept. King Inst. Prev. Med. Guindy* (1907), vol. 1 : 3 ; Wenyon, 1926, *Protozoology*, Vol. 1, pp. 596.
1910. *Trypanosoma rotatorium* Lebedeff, *Geburtst. Richard. Hertwings.* (Munchen), 1 : 397, Partim.

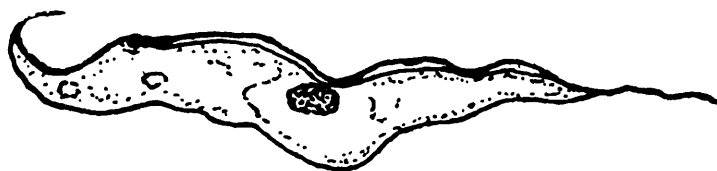


FIG. 12 10 μm

Fig. 12. Camera lucida drawing of *Trypanosoma inopinatum* in *Rana hexadactyla*

Type-host : *Rana esculenta* (Linn.)

Other hosts : *Rana hexadactyla* and *R. tigrina*.

Locality : South India (Patton, 1908*), Bankura, West Bengal.

Description : Body elongated with both the extremities tapering into fine processes ; measuring 30-34 μm in length and 6.0-9.0 μm in width. Cytoplasm is granular and vacuolated, particularly on the anterior half of the body ; myonemes present.

Nucleus, situated near the centre of the body, is usually elliptical with its axis parallel to the long axis of the body. It measures 3.0-6.0 μm × 2.0-3.0 μm.

The dot-like kinetoplast is located less than one half the distance from posterior extremity to nucleus.

Flagellum when present is very short, measuring 1.7 μm only. The undulating membrane is considerably narrow.

Life-cycle : Billet (1904) observed that this trypanosome was transmitted by the leech, *Helobdella algira*. The process of development in the leech was described by Brumpt (1906b). Present authors also succeeded to develop *T inopinatum* in the Indian Hirudinian host *Helobdella nociva*. In leech, vertebrate forms underwent some sequential changes to transform into invertebrate forms as follows : Trypanosome, in caecum losses flagellum, nucleus and kinetoplast divide, cell body elongates, longitudinal division occurs resulting in epimastigote and trypanosomal forms. Subsequently by means of repeated binary fission, numerous elongated slender short-membraned (crithidial) forms were produced which enter the proboscis sheath and transformed into metacyclic trypanosomes.

Remarks : Patton (1908) described one elongated trypanosome in *Rana hexadactyla* and *R. tigrina* from South India which he named as *Trypanosoma hendersoni*. Wenyon (1926) contended that this form was similar to *T inopinatum* Sergent and Sergent in all respects and Diamond (1958) synonymised *T hendersoni* with *T inopinatum*. *Trypanosoma inopinatum* is the only known pathogenic anuran trypanosome which causes "Red leg" disease among the common European frog *Rana esculenta*. But whether this trypanosome produces any disease in Indian frogs is not known.

6. *Trypanosoma ranarum* (Lankester) (Figs. 13a,b)

1843. *Trypanosoma sanguinis* Gruby, *Compt. Rend. Acad. Sc.*, **17** : 1134, Partim ; Danilewsky, 1885, *Biol. Zbl.*, **5** : 529 ; 1889, 'La parasitologie Comparee du Sang 1. Nouvelles recherches sur les Parasites du Sang des Oiseaux', Kharkoff, Partim.
1871. *Undulina ranarum* Lankester, *Q. Jl. microsc. Sci.*, **11** : 387.
1885. *Trypanosoma ranarum* Danilewsky, *Biol. Zbl.*, **5** : 529 ; 1889, La parasitologie comparee du Sadg. 1. Nouvelles recherches sur les parasites du Sang des Oiseaux, Kharkoff, Partim ; Damayanthi and Rao, 1979, *Proc. 2nd Natl. Cong. Parasitology*, p. 3 (Sec II).
1888. *Trypanosoma sanguinis ranarum* Shalashnikov, *Arch. Vet. Nauk. S. Peterburg.*, **1** : 65, Partim.
1889. *Trypanomonades ranarum* Danilewsky, La parasitologie du Sang des Oiseaux, Kharkoff, Partim.
1889. *Trypanosoma costatum* Danilewsky, *Ibid.*, Partim.
1889. *Trypanosoma costatum ranarum* Danilewsky, *Ibid*, Partim.
1901. *Trypanosoma rotatorium* Laveran and Mesnil, *C. r. Séanc. Soc. Biol.*, **53** : 678, Partim.
1906. *Trypanosoma loricatum* Franca and Athias, *Archs, Inst. Real. Bact.*, **1** : 127, Partim.
1906. *Trypanosoma loricatum vel costatum* Franca and Athias, *Ibid.*, Partim.
1907. *Trypanosoma clamatae* Stebbins, *Trans. Am. microsc. Soc.*, **27** : 25, Partim.

Type-host : *Rana clamitans*

Other hosts : *Rana esculenta*, *R. mugiens* and *R. pipiens*.

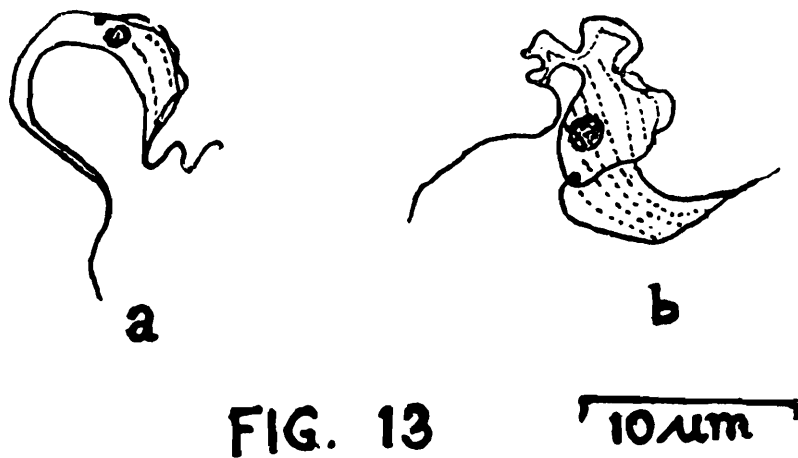


Fig. 13. Camera lucida drawings of *Trypanosoma loricatum* in *Rana tigrina*; a, Type I individual and b, Type II individual.

New Indian hosts : *Rana* sp. (?) and tadpoles (Damayanthi and Rao, 1979); *Rana tigrina*.

Locality : Warangal, Andhra Pradesh, India, and Bankura, West Bengal, India.

Description : The trypanosome is polymorphic designated as type I and II trypomastigotes. The body of type I is partially twisted on its longitudinal axis (Fig. 13a); flattened and widened. The body surface is smooth and is drawn out into a long tail-like appendage, posterior to kinetoplast. The body surface of Type II is costate and flattened (Fig. 13b); takes form of a spiral segment of a cornucopia with its pointed posterior extremity.

It measures $74.1 \mu\text{m}$ in length and $4.7 \mu\text{m}$ in width in Type I and $71.1 \mu\text{m}$ in length and $8.2 \mu\text{m}$ in width in Type II individuals.

Nucleus : Rounded or elliptical, located at anterior one-third of the body in type I, in middle-third of the body in type II. It measures $3.0 \times 2.5 \mu\text{m}$ in Type I and $3.6 \times 2.9 \mu\text{m}$ in Type II.

Kinetoplast : Large ; rectangular or elliptical ; located near the nucleus.

Flagellum : Measuring $13.5 \mu\text{m}$ in Type I and $13.2 \mu\text{m}$ in Type II which is less than one-half the body length. The undulating membrane is wide in both the forms.

The vectors and life-cycle is not known.

Remarks : In India, *Trypanosoma ranarum* was first recorded by Damayanthi and Rao (1979) in the heart muscle of some frogs of Warangal,

Andhra Pradesh. The occurrence of it was observed in tadpoles also but no morphometric measurements were given. They did not mention the name of the frogs and tadpoles. The flagellate was more prevalent in the heart than in the peripheral blood. The infection of this trypanosome caused remarkable change in muscle proteins of the heart of those frogs (Damayanthi and Rao, 1979). Authors have not noticed any visible disease symptoms in *Rana tigrina* investigated in West Bengal infected with *T. ranarum*.

7. ***Trypanosoma taprobanica*** Ray and Choudhury
(Figs. 11a, b & Plate V Figs. 6 & 7)

Ray and Choudhury, *Bull. zool. Surv. India* (In press).

Type-host : *Kaloula pulchra taprobanica* Parker.

Type-locality : Santaldi, Purulia District, West Bengal, India.

Prevalence : 14 out of 204 (6.9%) examples of *K. p. taprobanica* were positive for trypanosome.

Description (Figs. 11a, b & Plate V Figs. 6 & 7, Table 12, 13, 14) : The following description is based on the trypanosome observed in the peripheral blood of microhylid frogs, *Kaloula pulchra taprobanica*, collected from Purulia District, West Bengal, India.

The trypanosomes are monomorphic i. e., single form was observed throughout the three years of studies. They are very small, 'C' or 'S' shaped in configuration. Body curved, elongated with both the ends attenuated and pointed. The trypanosome measures 16.64 μm (15.0—18.5 μm) in length (excluding free flagellum) and 1.84 μm (1.5—2.0 μm) in width. Cytoplasm finely granular, homogeneous, stained light blue in Leishman. Body costae or striations were not found, Nucleus is small, rounded to oval, measuring 1.00 \times 0.56 μm ; stained red with Leishman and always situated on the posterior part of the body parallel to the body axis and close to the kinetoplast (NI=0.36).

Kinetoplast is a dot-like structure measuring 0.47 μm \times 0.48 μm and situated on the posterior part of the body very close to the nucleus (KI=3.65).

The flagellum emerges from the kinetoplast and traverses the whole body producing 4—6 prominent folds of the undulating membrane and finally leaves the body as a long free flagellum. The free flagellum is 8.3 μm in length and is about half the length of the body (FF/PA=0.49).

The method and site of reproduction in the vertebrate host has not been encountered in numerous samples of peripheral and heart blood and stained impression smears of heart, lung, liver, spleen and kidney examined.

Remarks : Ray and Choudhury (In press) described this haemoflagellate from a microhylid frog, *Kaloula pulchra taprobanica*. A perusal of the literature on Anuran trypanosomes revealed that there is no trypanosome being reported so far from the family Microhylidae. In this respect the present trypanosome is claimed to be reported for the first time from this family.

8. *Trypanosoma malabarica* sp. nov.
(Figs. 11c, d & Plate V Figs. 8 & 9)

Type-host : *Rana malabarica* Bibron

Type-locality : Volpoi, Goa, India

Additional host : *Rana limnocharis* Wiegmann

Prevalence : 7 out of 35 (20%) examples of *Rana malabarica* were found positive for the trypanosomes.

Description : The trypanosomes are monomorphic. The body is slender, elongated and very thin. It measures 24.07 μm in length (excluding free flagellum) and 3.96 μm in width. The body does not have a definite pattern of configuration but sometimes 'C' or 'S' shaped individuals may be found.

Cytoplasm is densely granulated, non-homogeneous, more granular in the middle portion and lightly granular in the anterior region. It stained dark blue. Body costae or striations are totally absent.

Nucleus is small, rounded to oval in structure having a central distinct karyosome. It measures 1.47 μm in length and 1.0 μm in width. The nucleus is situated on the posterior part of the body (NI=0.58) and stained red with Leishman and Giemsa stains.

Kinetoplast is a rounded dot-like structure measuring 0.67 μm X 0.50 μm ; stained deep blue with Leishman's stain and situated on the posterior end (KI=1.06) of the body.

The flagellum arises from the basal granule of the kinetoplast and traverses the whole body forming the conspicuous undulating membrane

(width=3.07 μm) with 4—7 folds and finally leaves the body as a long distinct free flagellum. The free flagellum measures 20.13 μm (18.00 μm —23.50 μm) in length and is more than three-fourth of the total body length (FF/PA=0.83).

The vector is unknown.

Type material : *Holotype* : Slide No. Z. S. I. Pt. 1972 is designated to a blood smear taken from *Rana malabarica* from Volpoi, Goa, India on 20.9.77, coll. R. Ray. *Paratype* : Slide No. Z. S. I. Pt. 1973 collection data same as holotype.

Remarks ; A review of the literature suggests that the shape and position of the kinetoplast have been used successfully for grouping trypanosomes of mammals (Hoare, 1964) and Urodels (Lehmann, 1959). The ratios of the various body measurements are relatively constant in a given species and this can be used for grouping or differentiating the species of Trypanosomes.

Mackerras and Mackerras (1961) clearly stated that the free flagellum when stained well with Leishman and properly oriented, may be used as a diagnostic feature.

Trypanosoma malabarica sp. nov. has also been found to infect *Rana limnocharis* in the same locality which is recorded as additional and second new host. The analysis of the morphometric measurements (Tables 15, 16) suggests that it is the same species with a little host induced variation.

In *R. limnocharis*, the haemoflagellates are a little larger in size, the width of the undulating membrane is greater and the nucleus is also little larger in comparison to the parasite of *Rana malabarica* (Table 16, 17).

Trypanosoma malabarica sp. nov. shows some similarities with *T. pipientis* Diamond, 1950 and *T. canadensis* Woo, 1969 in having long and slender body, nature of undulating membrane and presence of endosome. But it differs greatly from both of the trypanosomes referred above by the position of the nucleus which is situated in the posterior third of the body and the length of the free flagellum is always greater than the three-fourth of the body length. It also differs from the above mentioned trypanosomes by the granular nature of the cytoplasm and position of the kinetoplast which is close to the posterior extremity.

Considering the above morphometric parameters and a new anuran host from a new locality, the present haemoflagellate is considered as new to

science and the name *Trypanosoma malabarica* sp. nov. is attributed to its host's name.

9. ***Trypanosoma systoma* sp. nov.**
(Figs. 11e & Plate V Fig. 10)

Type-host : *Uperodon systoma* Schneider

Type-locality : Midnapore, West Bengal, India.

Prevalence : Out of 17 examples of *Uperodon systoma* examined, 4 (23.5) were found positive for this trypanosome.

Description : Trypanosomes are monomorphic. The body is slender, elongated with pointed anterior flagellar end and drawn out narrow posterior end. A depression or halo (Fig. 11e) is always found at the proximal extremity of the posterior end which is very very remarkable structure of these trypanosomes. Body measures 27.42 μm in length (excluding free flagellum) and 5.6 μm in width.

Cytoplasm is lightly granular, vacuolated and stained light blue with Leishman and Giemsa. Posterior end is more darker than the anterior. Costae or striations are not found.

Nucleus is oval or kidney shaped, situated on the posterior third of the body and parallel to the body axis. It measures $2.7 \times 1.62 \mu\text{m}$; stained deep red with Leishman and Giemsa. The nuclear membrane is well visible and there is no karyosome.

Kinetoplast is a rounded dot-like structure measuring 0.48 μm in diameter; stained deep blue and situated on the posterior fourth of the body (PK/PA=0.24) and it is very close to the nucleus (KI=4.91) (Table 18).

The flagellum emerges from the posterior end of the kinetoplast and traverses the whole body in the form of a very thin, well marked undulating membrane (width=1.36 μm) with four folds and finally leaves the body as a long free flagellum. The flagellum is well stained with Leishman and measuring 10.24 μm in length which is 1/3 of the total body length (FF/PA=0.37).

The method and site of multiplication are not yet known.

The vector is unknown.

Type material : *Holotype* : Slide No. Z. S. I. Pt 1974, is designated to a

blood smear taken from *Uperodon systoma*, from Midnapore, West Bengal, India on 15th August 1973. coll. A. K. Chandra. Paratype : On the same slide.

Remarks : So far the literature on amphibian trypanosome is concerned, there is no report of *Trypanosoma* infection in the family Microhylidae except one viz., *Trypanosoma taprobanica* Ray and Choudhury from *Kaloula pulchra taprobanica*, the authors communicated in this monograph.

The present species is well characterised by its narrow drawnout posterior end, position of the kinetoplast and presence of a depression or halo at the extremity of the posterior end which is very conspicuous.

The parasite under report greatly differs from *T taprobanica* sp. nov. the other hemoflagellate species from the family Microhylidae, in its size, position of the kinetoplast and length of the flagellum and other morphological details in general. Furthermore, this trypanosome resembles to some extent with *T karyozeukton* by the general body configuration particularly PK/PA, and undulating membrane (Table 19) but differs by possessing long flagellum, greater width of the body and without any surrounding halo of the kinetoplast and absence of chains of granules in between the kinetoplast and nucleus.

Summing the above facts and the morphological characteristics as displayed by the parasite described in this monograph, the authors contend to designate it a new name *Trypanosoma systoma* sp. nov.

PATHOGENESIS

Trypanosoma inopinatum is the only known pathogenic trypanosome which produce 'Red-leg' disease among the common European frog *Rana esculenta*. Brumpt (1906b) discovered that the Algerian strain of this trypanosome proved lethal to European green frogs when inoculated with infected blood or infected by leech feeding. The pathogenic effects were studied later by the same author (1924) revealing localized haemorrhages with swollen lymph glands and anaemia. The pathogenic ability of *T inopinatum* was also studied by Nöller (1917) and Franca (1912).

A good number of workers reported that *T rotatorium* is non-pathogenic (Creemers and Jadin, 1916 ; Lauter, 1960 ; Lebedeff, 1910 ; Mazza *et al.* (1927). However, Noller (1917) reported that heavy infections may lead to pathogenicity, especially in superinfections, resulting death with distinct amassing in the kidneys. A similar report was also given by

Reichenbach-Klinke and Elkan (1965) for Canadian frog infection, the pathogenicity manifesting itself as listlessness, food refusal and ultimate death. A similar observation was endorsed by the present authors in *Rana tigrina*. In course of investigation on anuran trypanosomes the present authors never noticed any pathological sign which could be correlated to trypanosomiasis in frogs. Damayanthi and Rao (1979) reported that the infection of *T. ranarum* causes remarkable changes in muscle protein of the heart of frogs in India. So far anurans are concerned, most of the above reports were just casual observations and thus could be confused with the symptoms of "Red-leg". The pathogenicity of *T. inopinatum* on the other hand, seems well established.

Pathogenicity is an exceedingly difficult phenomenon to establish, requiring repeated correlative and other studies.

HOST-PARASITE RELATIONSHIP

The relationship between the host and the parasite is sure to play an important role in the survival of the host as well as the parasite. It is an intricate process of adaptation and depends upon the compatibility between the host and the parasite. When a parasite invades a host various factors start to exercise their role and determine the degree of acceptance. A well adapted parasite must be able to maintain a condition of equilibrium where neither host nor parasite hampers each other's interest to the extent of lethal stage.

Since the trypanosomes feed exclusively at the expense of the fluid components of their host's body, they are detrimental to the host by depriving it of some of the substances required for the host's sustenance, by releasing toxic agents or otherwise interfering with its normal economy. The harmful effect of trypanosomes may result "Red-legged disease" in amphibians. But in many cases there is no evidence of any damage done by these parasites. In general, the course of all trypanosome infections is determined by the balance established between the aggressiveness of the parasite and the resistance put up by the hosts i.e., it all depends on the effectiveness of the immune response of the latter. In well-established associations, a mutual adjustment between the host and parasite is developed through age, enabling the two to live in apparent harmony, with the result that certain species or races of hosts have become tolerant to the infection.

The anuran trypanosomes are mostly harboured by aquatic and semi-aquatic cold-blooded hosts that offer a less species-specific environment

than do warm-blooded vertebrates. This means that the environment inhabited by anuran trypanosomes is on the one hand vastly variable, and on the other hand much less discrete, than that inhabited by avian or mammalian trypanosomes. Another important thing is that anuran trypanosomes are transmitted by sanguivorous leeches. The parasitic relationship between leeches, trypanosomes and aquatic Anura appears to be far more complex and intricate in its physiological and behavioural adaptations than the host-parasite system of mammalian trypanosomes, probably indicating a very old relationship (Bardsley and Harmsen, 1973).

The different aspects of host-parasite relationship were studied by a number of workers (Billet, 1904, Brumpt 1906b, Buttener and Bourcart 1955a ; Diamond, 1958 ; Barrow, 1953 ; Bardsley, 1969, 1972, Bardsley and Harmsen 1973, Ayala, 1971) abroad. In India, some works were done on these aspects. Pujati (1953) studied the transmission of *Trypanosoma rotatorium* through the leech *Placobdella ceylonica* in South India. Ray (1979a, b), Ray and Choudhury (1981) and Ray and Nandi (1978) studied the life-cycle, transmission and seasonal fluctuation of peripheral parasitaemia of *T rotatorium* from anurans of West Bengal, India.

The dynamics of the relationship between the trypanosome and the vertebrate host have recently become well documented and well modelled topic of research. Bardsley and Harmsen (1973) attempted to integrate existing scientific knowledge of anuran physiology, anuran ecology, the ecology of the invertebrate host and the behaviour and physiology of the anuran trypanosomes into a dynamic model of host parasite inter-relationship.

SUMMARY

1 In the present communication the authors described 9 species of *Trypanosoma* viz., *Trypanosoma rotatorium*, *T loricatum*, *T karyozeukton*, *T chattoni*, *T inopinatum*, *T ranarum*, *T taprobatica* sp. nov. *T malabarica* sp. nov. *T systoma* sp. nov. which have been recorded so far from India. Of these the last three have been christened with new species name.

2. *Trypanosoma rotatorium* (Mayer, 1843) has been communicated from seven Indian anuran hosts, viz., *Rana tigrina*, *R. Iimnocharis*, *R. cyanophlyctis*, *Bufo melanostictus*, *B. stomoticus*, *Rhacophorus maculatus* and *R. malabaricus* of which the last three have been added as new host records from the Indian subcontinent.

3. The authors successfully infected an Indian leech *Helobdella nociva* Harding with *T rotatorium* and studied all the developmental stages of the haemoflagellate in the invertebrate host.

4. *Trypanosoma loricatum* (Mayer, 1843) and *Trypanosoma karyozeukton* Dutton and Todd, 1903 have been reported for the first time from India and *Rana limnocharis* and *Rana hexadactyla* have been recorded as new hosts respectively for the two trypanosomes.
5. *Trypanosoma chattoni* Mathis and Léger, 1911a has been recorded from eight different anuran hosts from the same geographical locals (Nova Goa, India). The same species is also being communicated for the first time from Indian subcontinent and excepting *B. melanostictus* (type host) all the rest seven anuran amphibians are recorded as new hosts.
6. *Trypanosoma inopinatum* and *Trypanosoma ranarum* have been described in detail along with the life-cycle of the former,
7. *Trypanosoma taprobanica* from *Kaloula pulchra taprobanica*, *T. malabarica* from *Rana malabarica* and *T. systoma* from *Uperodon systoma* have been described as new species with detail morphometric measurements.
8. The pathogenicity of anuran trypanosomes has been discussed along with some comments on host-parasite relationship. A host-parasite list of the recorded species of Anuran trypanosomes in India has also been added at the end of the treatise.

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TABLE 1. Morphometric measurements of different forms of *T. rotatorium* in *Rana tigrina*. All measurements in micrometers.

| | Type I | | Type II | | Type III | | Type IV | |
|---------------------|--------|----------|---------|-----------|----------|-----------|---------|-----------|
| | Mean | Range | Mean | Range | Mean | Range | Mean | Range |
| PK | 1.33 | 1—2 | 3.5 | 3—4 | 1.64 | 0.8—2.4 | 5.42 | 2—11.2 |
| KN | 1.66 | 1.5—2 | 4.0 | 3.5—4.5 | 8.6 | 6.4—11.2 | 3.5 | 2.2—4.8 |
| PN | 3.0 | 2.5—4 | 7.5 | 7—8 | 10.33 | 8.2—13.2 | 10.44 | 7—13.7 |
| NA | 10.83 | 9—14 | 15.6 | 15.2—17 | 24.0 | 23—24 | 21.06 | 18—26.2 |
| PA | 13.83 | 11.5—18 | 23.5 | 23.2—24 | 29.0 | 28—30 | 31.52 | 29—34 |
| FF | 4.91 | 3—7 | 13.6 | 13.2—13.8 | 24.0 | 23.5—24.5 | 6.3 | 4—8.1 |
| BW | 2.26 | 2—2.5 | 3.1 | 3—3.2 | 4.0 | 3.5—4.5 | 7.78 | 6.4—9.7 |
| Kinetoplast Length | 0.66 | 0.5—0.75 | 0.8 | — | 0.8 | — | 0.8 | 0.5—1 |
| Width | 0.53 | 0.5—0.6 | 0.8 | — | 0.8 | — | 0.5 | — |
| KI | 1.77 | 1.6—2.0 | 1.8 | — | 2.5 | — | 3.25 | 1.4—5.48 |
| Nucleus Length | 1.90 | 1.8—2 | 2.4 | 2—2.4 | 2.4 | 2—2.4 | 2.2 | 2—2.5 |
| Breadth | 1.03 | 1—1.2 | 1.6 | 1—1.6 | 1.6 | 1.5—1.7 | 1.7 | 1.5—2 |
| NI | 0.27 | 0.2—0.3 | 0.4 | — | 0.3 | — | 0.51 | 0.26—0.76 |
| No. of undulations | 3.6 | 3—4 | 4 | 3—5 | 5 | 4—6 | 6.8 | 6—10 |
| Width of und. memb. | 1.2 | 1—1.5 | 1.5 | 1—2.5 | 2.0 | 1—2.5 | 2.38 | 2—2.7 |

Abbreviations : PK, the distance from the posterior end to the Kinetoplast ; KN, the distance from the Kinetoplast to the centre of the nucleus ; PN, the distance from the posterior end to the centre of the nucleus ; NA, the centre of the nucleus to the anterior end ; PA, the length of the body excluding the free flagellum, FF, the length of the free flagellum ; BW the maximum width excluding the undulating membrane, KI, the Kinetoplastic index ; NI, the nuclear index.

| | <i>Rana tigrina</i> | | | | <i>R. limnocharis</i> | | | |
|----------------------|---------------------|------|-------|-------|-----------------------|------|-------|-------|
| | M | SD | SE(±) | CV | M | SD | SE(±) | CV |
| PK | 5.42 | 3.50 | 1.57 | 64.57 | 0.83 | 0.31 | 0.10 | 37.34 |
| KN | 3.50 | 1.17 | 0.52 | 33.42 | 6.20 | 1.83 | 0.58 | 29.51 |
| PN | 10.44 | 2.60 | 1.17 | 24.90 | 7.03 | 1.81 | 0.57 | 25.74 |
| NA | 21.08 | 2.75 | 1.23 | 13.04 | 24.15 | 2.50 | 0.79 | 10.35 |
| PA | 31.52 | 1.95 | 0.87 | 6.18 | 31.18 | 3.08 | 0.97 | 9.87 |
| FF | 6.30 | 1.35 | 0.60 | 21.42 | 16.82 | 5.03 | 1.59 | 29.90 |
| TL | 37.82 | 3.30 | 1.47 | 27.60 | 48.00 | 8.11 | 2.56 | 39.77 |
| BW | 7.78 | 1.11 | 0.50 | 14.26 | 7.62 | 0.95 | 0.30 | 12.46 |
| Kinetoplast Length | 0.80 | 0.24 | 0.10 | 30.0 | 0.97 | 0.47 | 0.15 | 48.45 |
| Width | 0.50 | — | — | — | 0.47 | 0.04 | 0.01 | 8.51 |
| KI | 3.25 | 1.75 | 0.78 | 53.84 | 1.14 | 0.06 | 0.02 | 5.26 |
| Nucleus Length | 2.20 | 0.18 | 0.08 | 8.18 | 2.90 | 1.41 | 0.44 | 48.62 |
| Width | 1.70 | 0.24 | 0.10 | 14.11 | 1.65 | 0.39 | 0.12 | 23.63 |
| NI | 0.51 | 0.17 | 0.07 | 33.33 | 0.28 | 0.08 | 0.02 | 28.57 |
| No. of undl. | 6.80 | 1.72 | 0.77 | 25.29 | 6.00 | 1.18 | 0.39 | 19.66 |
| Width of undl. memb. | 2.38 | 0.24 | 0.11 | 10.08 | 2.66 | 0.94 | 0.31 | 35.33 |

Abbreviations as in Table 1.

TABLE 5. Comparative morphometric measurements of *Trypanosoma rotatorium* (Mayer, 1843) in *Rana tigrina*, *R. limnocharis*, *R. cyanophlyctis*, *Bufo melanostictus*, *B. stomaticus*, *Rhacophorus maculatus*, *Rhacophorus malabaricus*. All linear measurements in micrometer. M, represents Mean, SD = Standard deviation, SE = Standard error of the mean, CV = Coefficient of variation.

| <i>R. cyanophlyctis</i> | | | | <i>Bufo melanostictus</i> | | | | <i>B. stomaticus</i> | |
|-------------------------|------|-------|-------|---------------------------|------|-------|-------|----------------------|------|
| M | SD | SE(±) | CV | M | SD | SE(±) | CV | M | SD |
| 1.66 | 0.20 | 0.09 | 12.04 | 1.60 | 0.73 | 0.32 | 45.62 | 3.53 | 2.69 |
| 9.90 | 3.73 | 1.67 | 37.67 | 6.20 | 1.50 | 0.67 | 24.19 | 11.81 | 3.38 |
| 11.60 | 3.59 | 1.61 | 30.94 | 7.80 | 1.53 | 0.68 | 19.61 | 15.35 | 4.55 |
| 16.10 | 2.24 | 1.0 | 13.91 | 23.44 | 0.84 | 0.37 | 3.58 | 17.66 | 3.18 |
| 27.7 | 2.70 | 1.21 | 9.74 | 31.24 | 0.98 | 0.44 | 3.13 | 33.07 | 4.92 |
| 20.40 | 2.87 | 1.28 | 14.06 | 11.44 | 1.84 | 0.82 | 16.08 | 16.00 | 2.97 |
| 48.10 | 5.57 | 2.49 | 23.80 | 42.68 | 2.82 | 1.26 | 19.21 | 49.07 | 7.89 |
| 6.02 | 0.53 | 0.23 | 8.80 | 8.50 | 1.14 | 0.51 | 13.41 | 10.05 | 1.16 |
| 1.00 | — | — | — | 0.84 | 0.18 | 0.08 | 21.42 | 0.63 | 0.23 |
| 0.5 | — | — | — | 0.46 | 0.04 | 0.02 | 8.69 | 0.47 | 0.04 |
| 1.19 | 0.08 | 0.03 | 6.72 | 1.28 | 0.18 | 0.08 | 14.06 | 1.21 | 0.17 |
| 3.20 | 0.92 | 0.41 | 28.75 | 3.70 | 2.22 | 0.99 | 60.00 | 8.05 | 4.16 |
| 1.48 | 0.39 | 0.17 | 26.35 | 1.80 | 0.50 | 0.22 | 27.70 | 2.18 | 0.24 |
| 0.75 | 0.36 | 0.16 | 48.0 | 0.33 | 0.07 | 0.03 | 21.21 | 0.74 | 0.22 |
| 6.20 | 1.16 | 0.52 | 18.70 | 7.00 | 1.67 | 0.75 | 23.8 | 5.75 | 0.68 |
| 1.90 | 0.02 | 0.08 | 10.52 | 2.44 | 0.38 | 0.17 | 15.57 | 1.97 | 0.52 |

TABLE 5. Comparative morphometric measurements of *Trypanosoma rotatorium* (Mayer, 1843) in *Rana tigrina*, *R. limnocharis*, *R. cyanophlyctis*, *Bufo melanostictus*, *B. stomaticus*, *Rhacophorus maculatus*, *Rhacophorus malabaricus*. All linear measurements in micrometer. M, represents Mean, SD=Standard deviation, SE=Standard error of the mean, CV=Coefficient of variation.

| M | <i>R. cyanophlyctis</i> | | | <i>Bufo melanostictus</i> | | | <i>B. stomaticus</i> | | | <i>Rhacophorus maculatus</i> | | | | <i>Rhacophorus malabaricus</i> | | | | | |
|-------|-------------------------|-------|-------|---------------------------|------|-------|----------------------|-------|------|------------------------------|-------|-------|------|--------------------------------|-------|-------|------|-------|-------|
| | SD | SE(±) | CV | M | SD | SE(±) | CV | M | SD | SE(±) | CV | M | SD | SE(±) | CV | M | SD | SE(±) | CV |
| 1.66 | 0.20 | 0.09 | 12.04 | 1.60 | 0.73 | 0.32 | 45.62 | 3.53 | 2.69 | 0.95 | 76.20 | 2.10 | 1.15 | 0.36 | 54.76 | 0.96 | 0.29 | 0.12 | 30.20 |
| 9.90 | 3.73 | 1.67 | 37.67 | 6.20 | 1.50 | 0.67 | 24.19 | 11.81 | 3.38 | 1.16 | 27.77 | 10.57 | 1.98 | 0.62 | 18.73 | 6.83 | 3.51 | 1.44 | 51.39 |
| 11.60 | 3.59 | 1.61 | 30.94 | 7.80 | 1.53 | 0.68 | 19.61 | 15.35 | 4.55 | 1.61 | 29.64 | 12.67 | 2.73 | 0.86 | 21.54 | 7.80 | 3.35 | 1.37 | 42.94 |
| 16.10 | 2.24 | 1.0 | 13.91 | 23.44 | 0.84 | 0.37 | 3.58 | 17.66 | 3.18 | 1.13 | 18.0 | 16.32 | 2.04 | 0.64 | 12.50 | 21.50 | 3.39 | 1.39 | 15.75 |
| 27.7 | 2.70 | 1.21 | 9.74 | 31.24 | 0.98 | 0.44 | 3.13 | 33.07 | 4.92 | 1.74 | 14.87 | 28.99 | 3.79 | 1.19 | 13.07 | 28.71 | 3.53 | 1.44 | 12.29 |
| 20.40 | 2.87 | 1.28 | 14.06 | 11.44 | 1.84 | 0.82 | 16.08 | 16.00 | 2.97 | 0.99 | 17.43 | 12.28 | 3.11 | 0.98 | 25.38 | 20.08 | 1.90 | 0.77 | 9.46 |
| 48.10 | 5.57 | 2.49 | 23.80 | 42.68 | 2.82 | 1.26 | 19.21 | 49.07 | 7.89 | 2.73 | 32.30 | 41.27 | 6.90 | 2.17 | 38.45 | 48.79 | 5.43 | 2.21 | 21.75 |
| 6.02 | 0.53 | 0.23 | 8.80 | 8.50 | 1.14 | 0.51 | 13.41 | 10.05 | 1.16 | 0.41 | 11.54 | 7.25 | 2.16 | 0.68 | 29.79 | 8.25 | 2.49 | 1.02 | 30.18 |
| 1.00 | — | — | — | 0.84 | 0.18 | 0.08 | 21.42 | 0.63 | 0.23 | 0.08 | 36.50 | 0.47 | 0.04 | 0.01 | 8.50 | 0.58 | 0.18 | 0.07 | 31.03 |
| 0.5 | — | — | — | 0.46 | 0.04 | 0.02 | 8.69 | 0.47 | 0.04 | 0.01 | 8.51 | 0.47 | 0.04 | 0.01 | 8.50 | 0.50 | — | — | — |
| 1.19 | 0.08 | 0.03 | 6.72 | 1.28 | 0.18 | 0.08 | 14.06 | 1.21 | 0.17 | 0.06 | 14.04 | 1.19 | 0.09 | 0.02 | 7.56 | 1.19 | 0.13 | 0.05 | 10.92 |
| 3.20 | 0.92 | 0.41 | 28.75 | 3.70 | 2.22 | 0.99 | 60.00 | 8.05 | 4.16 | 1.47 | 48.94 | 9.30 | 1.87 | 0.59 | 20.10 | 6.33 | 4.02 | 1.65 | 63.50 |
| 1.48 | 0.39 | 0.17 | 26.35 | 1.80 | 0.50 | 0.22 | 27.70 | 2.18 | 0.24 | 0.08 | 11.0 | 1.81 | 0.47 | 0.14 | 25.96 | 1.75 | 0.38 | 0.15 | 21.71 |
| 0.75 | 0.36 | 0.16 | 48.0 | 0.33 | 0.07 | 0.03 | 21.21 | 0.74 | 0.22 | 0.08 | 29.72 | 0.79 | 0.16 | 0.05 | 20.77 | 0.37 | 0.20 | 0.08 | 54.05 |
| 6.20 | 1.16 | 0.52 | 18.70 | 7.00 | 1.67 | 0.75 | 23.8 | 5.75 | 0.68 | 0.27 | 11.82 | 5.70 | 0.90 | 0.28 | 15.78 | 6.83 | 0.68 | 0.28 | 9.95 |
| 1.90 | 0.02 | 0.08 | 10.52 | 2.44 | 0.38 | 0.17 | 15.57 | 1.97 | 0.52 | 0.18 | 26.39 | 1.43 | 0.36 | 0.11 | 25.17 | 1.83 | 0.55 | 0.22 | 30.05 |

TABLE 6. Morphometric measurements of *T. loricatum* in *Rana limnocharis*. All linear measurements in micrometer. M. represent Mean, SD=Standard deviation. SE= Standard error of the mean, CV=Coefficient of variation.

| | Type—I (Slender form) | | | | Type—II (Broad form) | | | |
|----------------------|-----------------------|------|-------|-------|----------------------|------|-------|-------|
| | M | SD | SE(±) | CV | M | SD | SE(±) | CV |
| PK | 3.50 | 0.65 | 0.24 | 18.57 | 6.70 | 0.92 | 0.41 | 13.73 |
| KN | 0.9 | 0.17 | 0.06 | 18.88 | 1.32 | 0.19 | 0.08 | 14.39 |
| PN | 4.4 | 0.77 | 0.29 | 17.50 | 7.82 | 1.16 | 0.52 | 14.83 |
| NA | 11.85 | 1.21 | 0.46 | 10.21 | 12.88 | 1.01 | 0.45 | 7.84 |
| PA | 16.25 | 0.62 | 0.23 | 3.81 | 20.76 | 0.93 | 0.41 | 4.47 |
| FF | 5.85 | 2.51 | 0.95 | 42.9 | 4.96 | 1.01 | 0.45 | 20.36 |
| BW | 2.34 | 0.35 | 0.13 | 14.95 | 6.70 | 0.50 | 0.22 | 7.46 |
| KinetoPlast : Length | 0.41 | 0.03 | 0.01 | 7.31 | 0.48 | 0.04 | 0.01 | 8.33 |
| Width | 0.42 | 0.03 | 0.01 | 7.31 | 0.48 | 0.04 | 0.01 | 8.33 |
| KI | 4.97 | 0.69 | 0.26 | 13.88 | 6.0 | 1.08 | 0.48 | 18.0 |
| Nucleus : Length | 1.01 | 0.20 | 0.07 | 19.8 | 1.40 | 0.20 | 0.08 | 14.28 |
| Width | 0.5 | — | — | — | 1.04 | 0.08 | 0.03 | 7.69 |
| NI | 0.37 | 3.10 | 0.03 | 20.0 | 0.61 | 0.13 | 0.05 | 21.31 |

Abbreviations : As in Table 1.

TABLE 7. Body ratios* of *T. loricatum* in *Rana limnocharis*.

| | PK/PA | PN/PA | KN/PN | PK/PN | BW/PA | FF/PA |
|---------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Type I | 0.21 (0.14-0.29) | 0.26 (0.17-0.35) | 0.20 (0.16-0.25) | 0.79 (0.75-0.83) | 0.14 (0.09-0.19) | 0.35 (0.18-0.60) |
| Type II | 0.31 (0.27-0.39) | 0.37 (0.31-0.45) | 0.19 (0.13-0.23) | 0.85 (0.82-0.92) | 0.31 (0.28-0.37) | 0.23 (0.17-0.29) |

*Mean followed by range.

TABLE 8. Morphometric measurements of *T. karyozeukton* in *Rana hexadactyla*. All measurements in micron. M=Mean, S.D.=standard deviation, S.E.=standard error of the mean and C.V.=Coefficient of variation.

| | M | SD | SE | CV |
|-------------------------|-------|------|------|-------|
| PK | 9.34 | 0.66 | 0.29 | 7.06 |
| KN | 10.46 | 0.40 | 0.18 | 3.82 |
| PN | 19.80 | 0.97 | 0.43 | 4.89 |
| NA | 24.84 | 0.42 | 0.19 | 1.69 |
| PA | 44.64 | 1.31 | 0.59 | 2.93 |
| FF | 9.7 | 0.18 | 0.08 | 1.85 |
| Bw | 2.64 | 0.19 | 0.08 | 7.19 |
| Kinetoplast length | 0.7 | 0.16 | 0.07 | 22.85 |
| width | 0.5 | — | — | — |
| Nucleus Length | 2.34 | 0.24 | 0.10 | 10.25 |
| Width | 1.24 | 0.16 | 0.07 | 12.90 |
| Width of undl. membrane | 1.38 | 0.11 | 0.05 | 7.97 |

Abbreviation as in Table 1

TABLE 9. Body ratios* of *T. karyozeukton* Dutton and Todd 1903 in *Rana hexadactyla*, an Indian frog.

| PK/PA | PN/PA | KN/PN | PK/PN | BW/PA | FF/PA |
|-------------|-------------|-------------|-------------|-------------|-------------|
| 0.20 | 0.43 | 0.52 | 0.46 | 0.05 | 0.21 |
| (0.19—0.22) | (0.43—6.45) | (0.51—0.54) | (0.45—0.48) | (0.05—0.06) | (0.20—0.22) |

*Mean followed by range.

TABLE 10. Comparative morphological parameters of small forms of *T. karyozeukton* Dutton *et. al.*, 1907, and the same species recorded from *R. hexadactyla* in the present study. All measurements in microns.

| | From Dutton <i>et. al.</i> , 1907 | | From the present study | |
|---------------------|-----------------------------------|---------|------------------------|---------|
| | Range | Average | Range | Average |
| PK | 9—20 | — | 8.5—10.5 | 9.34 |
| KN | 3.8—8.5 | — | 10—11 | 10.46 |
| PN | 12.8—28.5 | — | 18.5—21.5 | 19.8 |
| NA | 20.7—38.0 | — | 24.5—25.5 | 24.84 |
| PA | 31.0—45.0 | — | 43—47 | 44.64 |
| FF | 18.5—23.0 | — | 9.5—10 | 9.70 |
| TL | 59.5—87.0 | — | 52.5—57 | 54.34 |
| BW | — | — | 2.5—3 | 2.64 |
| Kinetoplast Length | — | — | 0.5—0.9 | 0.7 |
| Width | — | — | — | 0.5 |
| KI | — | — | 1.85—1.95 | 1.89 |
| Nucleus Length | 2.2—3.5 | — | 2—2.7 | 2.34 |
| Width | 1.6—2.7 | — | 1—1.5 | 1.24 |
| NI | — | — | 0.75—0.85 | 0.79 |
| No. of Wndl. | — | — | 4—7 | 5.4 |
| Width of undl. mem. | — | — | 1.2—1.5 | 1.38 |

Abbreviations as in Table 1.

TABLE 11. Comparative morphometric measurements of *T. chattoni* in *Bufo melanostictus*, *B. stomaticus*, *Rana limnocharis*, *R. cyanophlyctis* and *Rhacophorus maculatus*. All linear measurements in micrometer. M=represents Mean, SD=Standard deviation, SE=Standard error of the mean and CV=Coefficient of variation.

| | <i>Bufo melanostictus</i> | | | | <i>Bufo stomaticus</i> | | | |
|---------------------------------|---------------------------|-------|-------|-------|------------------------|-------|-------|-------|
| | M | SD | SE | CV | M | SD | SE | CV |
| Body diameter | 21.83 | 1.17 | 0.37 | 5.35 | 26.15 | 1.59 | 0.50 | 6.08 |
| Body area (um ²) | 373.64 | 44.85 | 14.19 | 12.0 | 467.8 | 58.91 | 18.68 | 12.59 |
| Nuclear diameter | 6.62 | 0.71 | 0.22 | 10.72 | 6.37 | 0.42 | 0.13 | 6.59 |
| Nuclear area (um ²) | 29.26 | 5.87 | 1.85 | 20.06 | 27.11 | 2.15 | 0.68 | 7.93 |
| Length of flagellum | 4.59 | 1.49 | 0.47 | 32.46 | 4.75 | 1.07 | 0.34 | 22.52 |
| Kinetoplast | | | | | | | | |
| Length | 0.51 | 0.17 | 0.05 | 33.33 | 0.55 | 0.16 | 0.05 | 29.09 |
| Width | 0.45 | 0.05 | 0.01 | 11.11 | 0.48 | 0.03 | 0.01 | 6.25 |

TABLE 12. Morphometric measurements of *Trypanosoma taprobanica* sp. nov. in *Kaloula pulchra taprobanica* Parker. All linear measurements in micrometer. M. indicates Mean, SD=Standard deviation, SE=Standard error of the mean and CV=Coefficient of variation.

| | M | SD | SE | CV |
|--------------------|-------|------|------|-------|
| PK | 3.20 | 0.24 | 0.10 | 7.50 |
| KN | 1.30 | 0.40 | 0.17 | 30.76 |
| PN | 4.50 | 0.54 | 0.24 | 12.00 |
| NA | 12.14 | 1.12 | 0.50 | 9.22 |
| PA | 16.64 | 1.42 | 0.63 | 8.53 |
| FF | 8.30 | 0.18 | 0.08 | 2.16 |
| BW | 1.84 | 0.20 | 0.09 | 10.86 |
| Kinetoplast Length | 0.47 | 0.04 | 0.01 | 8.51 |
| Width | 0.48 | 0.04 | 0.01 | 8.51 |
| KI | 3.65 | 0.66 | 0.29 | 18.08 |
| Nucleus Length | 1.00 | — | — | — |
| Width | 0.59 | 0.12 | 0.05 | 21.42 |
| NI | 3.36 | 0.04 | 0.01 | 11.11 |

KI=Kinetoplastic index, NI=Nuclear index.
Abbreviations as in Table 1.

| <i>Rana limnocharis</i> | | | | <i>Rana cyanophlyctis</i> | | | | <i>Rhacophorus maculatus</i> | | | |
|-------------------------|-------|-------|-------|---------------------------|-------|-------|-------|------------------------------|-------|-------|-------|
| M | SD | SE | CV | M | SD | SE | CV | M | SD | SE | CV |
| 20.0 | 3.12 | 0.98 | 15.60 | 20.70 | 1.56 | 0.59 | 7.53 | 19.74 | 2.38 | 0.79 | 12.05 |
| 341.57 | 53.27 | 16.85 | 15.59 | 323.28 | 43.52 | 16.48 | 13.46 | 276.46 | 65.30 | 21.76 | 23.62 |
| 4.65 | 0.88 | 0.28 | 18.92 | 4.70 | 0.35 | 0.13 | 7.44 | 3.81 | 0.59 | 0.19 | 15.85 |
| 16.47 | 5.41 | 1.71 | 32.84 | 15.14 | 1.80 | 0.68 | 11.88 | 9.04 | 2.38 | 0.79 | 26.32 |
| 5.50 | 1.26 | 0.40 | 22.90 | 5.17 | 0.69 | 0.26 | 13.34 | 5.66 | 1.24 | 0.71 | 21.90 |
| 0.47 | 0.10 | 0.03 | 21.27 | 0.45 | 0.05 | 0.01 | 11.11 | 0.31 | 0.05 | 0.01 | 16.21 |
| 0.43 | 0.07 | 0.02 | 16.27 | 0.38 | 0.06 | 0.02 | 15.78 | 0.31 | 0.05 | 0.01 | 16.21 |

TABLE 13. Comparative body ratio* of *T. taprobanica*, Type I slender form of *T. loricatum* and Juvenile form of *T. rotatorium*.

| Name of the parasite | PK/PA | PN/PA | KN/PN | PK/PN | BW/PA | FF/PA |
|------------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| <i>T. taprobanica</i> sp. nov. | 0.18 (0.18-0.20) | 0.26 (0.24-0.30) | 0.28 (0.22-0.36) | 0.71 (0.63-0.77) | 0.10 (0.09-0.13) | 0.49 (0.44-0.55) |
| <i>T. loricatum</i> (Mayer, 1843) | 0.21 (0.14-0.29) | 0.26 (0.17-0.35) | 0.20 (0.16-0.25) | 0.79 (0.75-0.83) | 0.14 (0.09-0.16) | 0.35 (0.18-0.60) |
| <i>T. rotatorium</i> (Mayer, 1843) | 0.08 (0.06-0.1) | 0.19 (0.17-0.21) | 0.56 (0.5-0.6) | 0.43 (0.4-0.5) | 0.16 (0.13-0.20) | 0.35 (0.25-0.41) |

*Mean followed by range.

TABLE 14. Comparative morphological measurements of *T. taprobanica* sp. nov. with slender form of *T. loricatum* and Juvenile form of *T. rotatorium*. All measurements in micrometers.

| | <i>T. taprobanica</i> | | <i>T. loricatum</i> | | <i>T. rotatorium</i> | |
|-------------|-----------------------|-------|---------------------|-------|----------------------|-------|
| | Range | Mean | Range | Mean | Range | Mean |
| PK | 3—3.5 | 3.2 | 2.5—4.5 | 3.5 | 1—2 | 1.33 |
| KN | 1—2 | 1.3 | 0.5—1 | 0.9 | 1.5—2 | 1.66 |
| PN | 4—5.5 | 4.5 | 3—5.5 | 4.4 | 2.5—4 | 3.0 |
| NA | 10.5—13.5 | 12.14 | 10—14 | 11.85 | 9—14 | 10.83 |
| PA | 15—18.5 | 16.64 | 15.3—17 | 16.25 | 11.5—18.0 | 13.83 |
| FF | 8—8.5 | 8.3 | 3—10 | 5.85 | 3—7 | 4.92 |
| BW | 1.5—2 | 1.84 | 1.6—2.8 | 2.34 | 2—2.5 | 2.26 |
| Kinetoplast | | | | | | |
| Length | 0.4—0.5 | 0.47 | 0.4—0.5 | 0.41 | 0.50—0.75 | 0.66 |
| Width | 0.4—0.5 | 0.48 | 0.4—0.5 | 0.42 | 0.5—0.6 | 0.53 |
| KI | 2.75—4 | 3.65 | 4—6 | 4.97 | 1.66—2.0 | 1.77 |
| Nucleus | | | | | | |
| Length | — | 1.00 | 0.9—1.5 | 1.01 | 1.8—2.0 | 1.90 |
| Width | 0.5—0.3 | 0.56 | — | 0.5 | 1—1.2 | 1.03 |
| NI | 0.32—0.42 | 0.36 | 0.21—0.55 | 0.37 | 0.20—0.38 | 0.27 |

Abbreviations : PK, posterior end to the kinetoplast ; KN, Kinetoplast to the centre of the nucleus ; PN, Posterior end to the centre of the nucleus NA, Centre of the nucleus to the anterior end ; PA, posterior end to the anterior end ; FF, length of free flagellum ; BW, Body width, KI, Kinetoplastic index ; NI, Nucleus index.

TABLE 15. Morphological measurements of *Trypanosoma malabarica* sp. nov. in *Rana malabarica*. Linear measurements in micrometer. M indicates Mean, SD= standard deviation, SE=Standard error of the mean and CV=Coefficient of variation.

| | M | SD | SE | CV |
|--------------------|-------|------|------|-------|
| PK | 0.54 | 0.36 | 0.36 | 66.60 |
| KN | 7.61 | 3.06 | 0.96 | 40.00 |
| PN | 8.15 | 3.39 | 1.07 | 41.59 |
| NA | 15.92 | 3.36 | 1.15 | 21.10 |
| PA | 24.07 | 1.34 | 0.42 | 5.56 |
| FF | 20.13 | 1.56 | 0.49 | 7.74 |
| TL | 44.60 | 1.54 | 0.48 | 3.45 |
| BW | 4.96 | 0.48 | 0.15 | 12.12 |
| Kinetoplast Length | 0.67 | 0.26 | 0.08 | 38.80 |
| Width | 0.50 | — | — | — |
| KI | 1.06 | 0.05 | 0.01 | 4.71 |
| Nucleus Length | 1.47 | 0.38 | 0.12 | 25.85 |
| Width | 1.00 | — | — | — |
| NI | 0.58 | 0.34 | 0.10 | 58.62 |
| No. of undulation | 5.2 | 1.07 | 0.34 | 20.57 |

Abbreviations as in Table 1 and 14.

TABLE 16. Comparative morphometric measurements of *Trypanosoma malabarica* sp. nov. in *Rana malabarica* and *R. limnocharis*. Linear measurements in micrometers.

| | <i>Rana malabarica</i> | | <i>Rana limnocharis</i> | |
|---------------------|------------------------|-------|-------------------------|-------|
| | Range | Mean | Range | Mean |
| PK | 0.5—1.5 | 0.54 | 0.5—1 | 0.65 |
| KN | 3—12.5 | 7.61 | 4—12 | 6.64 |
| PN | 3—13.5 | 8.15 | 4.5—13 | 7.29 |
| NA | 10—18.2 | 15.92 | 20—25.5 | 22.38 |
| PA | 22.5—27 | 24.07 | 25.3—33 | 29.77 |
| FF | 18—23.5 | 20.13 | 17—23.5 | 20.25 |
| BW | 3—5 | 3.96 | 2.5—6 | 3.93 |
| Kinetoplast Length | 0.5—1.2 | 0.67 | 0.3—0.5 | 0.44 |
| Width | — | 0.5 | 0.3—0.5 | 0.42 |
| KI | 1—1.14 | 1.06 | 1.05—1.23 | 1.10 |
| Nucleus Length | 1—2.2 | 1.47 | 2.5—4.5 | 3.3 |
| Width | — | 1.0 | 1.5—2.2 | 1.88 |
| NI | 0.14—1.35 | 0.58 | 0.21—0.65 | 0.32 |
| Width of undl. mem. | 1.5—4 | 3.07 | 1—1.5 | 1.39 |

Abbreviations as in Table 1 & 14.

TABLE 17. Comparative body ratios* of *T. malabarica* sp. nov. in two different hosts viz., *Rana malabarica* and *R. limnocharis*.

| Name of hosts | PK/PA | PN/PA | KN/PN | PK/PN | BW/PA | FF/PA |
|-------------------------|---------------------|---------------------|---------------------|--------------------|---------------------|---------------------|
| <i>Rana malabarica</i> | 0.02 (0.02—0.05) | 0.33 (0.12—0.57) | 0.93 (0.87—1) | 0.05 (0.—0.12) | 0.16 (0.13—0.22) | 0.83 (0.78—1) |
| <i>Rana limnocharis</i> | 0.01 (0.01—0.39) | 0.23 (0.17—0.39) | 0.90 (0.88—0.94) | 0.07 (0.1—0.19) | 0.12 (0.07—0.18) | 0.67 (0.61—0.79) |

TABLE 18. Morphometric measurements of *Trypanosoma systoma* sp. nov. in *Uperodon systoma*. All linear measurements in micrometers. M indicates Mean, SD= Standard deviation, SE=Standard error of the mean and CV=Coefficient of variation.

| | M | SD | SE | CV |
|--------------------|-------|------|------|-------|
| PK | 6.62 | 0.29 | 0.13 | 4.38 |
| KN | 1.72 | 0.23 | 0.10 | 13.37 |
| PN | 8.34 | 0.36 | 0.16 | 4.31 |
| NA | 19.08 | 2.8 | 1.30 | 14.67 |
| PA | 27.42 | 3.0 | 1.34 | 10.94 |
| FF | 10.24 | 0.22 | 0.10 | 2.14 |
| BW | 5.60 | 0.66 | 0.29 | 11.78 |
| Kinetoplast Length | 0.48 | 0.04 | 0.01 | 8.33 |
| Width | 0.48 | 0.04 | 0.01 | 8.33 |
| KI | 4.91 | 0.49 | 0.22 | 9.97 |
| Nucleus Length | 2.7 | 0.40 | 0.17 | 14.81 |
| Width | 1.62 | 0.19 | 0.08 | 11.72 |
| NI | 0.44 | 0.07 | 0.03 | 15.90 |

Abbreviations as in the Table 1 and 14.

TABLE 19. Comparative body ratios* of *T. systoma* sp. nov. with *T. karyozeukton* recorded in the present study.

| Name of parasite | PK/PA | PN/PA | KN/PN | PK/PN | BW/PA | FF/PA |
|---|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| <i>T. systoma</i> sp. nov. | 0.24 (0.22-0.28) | 0.30 (0.27-0.37) | 0.20 (0.18-0.24) | 0.78 (0.75-0.81) | 0.20 (0.17-0.23) | 0.37 (0.33-0.46) |
| <i>T. karyozeukton</i> Dutton and Todd, 1903 | 0.20 (0.19-0.22) | 0.43 (0.43-0.45) | 0.52 (0.51-0.54) | 0.46 (0.45-0.48) | 0.05 (0.05-0.06) | 0.21 (0.20-0.22) |

*Mean followed by range.

TABLE 20. Host-parasite list of recorded species of Anuran trypanosomes in India.

| Host's Name | Parasite | References |
|---------------------------------------|---------------------------------------|--|
| 1. <i>Bufo melanostictus</i> | <i>Trypanosoma rotatorium</i> | Wenyon, 1926 ; Ray, 1979 a, b, 1980 |
| | <i>T. chattoni</i> | Ray, 1979 b, 1980 |
| 2. <i>Bufo stomaticus</i> | <i>T. rotatorium</i> | Ray, 1979 a, b, 1980 |
| | <i>T. chattoni</i> | Ray, 1979, a, b, 1980 |
| 3. <i>Kaloula pulchra taprobanica</i> | <i>T. taprobanica</i> sp. Nov. | Ray & Choudhury (In press) |
| 4. <i>Microhyla ornata</i> | <i>T. hattoni</i> | Ray 1979 b. |
| 5. <i>Rana cyanophlyctis</i> | <i>T. rotatorium</i> | Pujati, 1953 ; Ray, 1979 a.b, 1980 |
| | <i>T. chattoni</i> | Ray, 1979 a.b, 1980 |
| 6. <i>Rana hexadactyla</i> | <i>T. karyozeukton</i> | Ray, 1979 b, 1980 |
| | <i>T. inopinatum (=hendersoni)</i> | Patton, 1908 |
| 7. <i>Rana limnocharis</i> | <i>T. rotatorium</i> | Berestneff, 1903 ; Patton, 1908 ; Scott, 1926, 1927 ; Ray, 1979 a.b ; Ray and Nandi 1978 |
| | <i>T. chattoni & T. loricatum</i> | Ray 1979 b, 1980 |
| 8. <i>Rana malabarica</i> | <i>T. malabarica</i> sp. Nov. | Ray, 1979 b |
| 9. <i>Rana tigrina</i> | <i>T. rotatorium</i> | Berestheff, 1903 ; patton, 1908 ; Scott, 1926 ; 1927 ; Pujati, 1953 ; Ray, 1979 a, b, 1980 ; Ray & Choudhury, 1980, 1981 |
| | <i>T. inopinatum (=hendersoni)</i> | Patton, 1908 |
| | <i>T. chattoni</i> | Ray, 1979 b, 1980 |
| *10. <i>Rana</i> sp. and tadpoles | <i>T. ranarum</i> | Damayanthi & Rao, 1979 |
| 11. <i>Rhacophorus maculatus</i> | <i>T. rotatorium</i> | Ray, 1979 a, b, 1980 |
| | <i>T. chattoni</i> | Ray, 1979 b, 1980 |
| 12. <i>Rhacophorus malabaricus</i> | <i>T. rotatorium</i> | Ray, 1979 b |
| 13. <i>Uperodon systema</i> | <i>T. systema</i> sp. nov. | Ray, 1979 b |

Uninfected hosts : *Rana tigrina crassa*, *R. limnocharis syhadrensis*, *R. beddomii*, *R. erythraca*, *R. brevipalmata*, *Bufo andersoni*, *B. himalayanus*, *Kaloula pulchra*.

*Name of the host species has not been mentioned by the original authors

PLATE I

Figs. 1—7. Photomicrographs of *Trypanosoma rotatorium* from 4 anuran hosts.

1. Type I (Juvenile form) in *Rana tigrina* X 1500
2. Type II (slender form) in the same host X 1000
3. Type III (Flat leaf-like form) in *Rana tigrina* X 1000
4. Type IV (Larger compact form) in the same host X 2500
5. *T. rotatorium* in *Rana limnocharis* X 1165
6. *T. rotatorium* in *Rana cyanophlyctis* X 1075
7. *T. rotatorium* in *Bufo melanostictus* X 1450

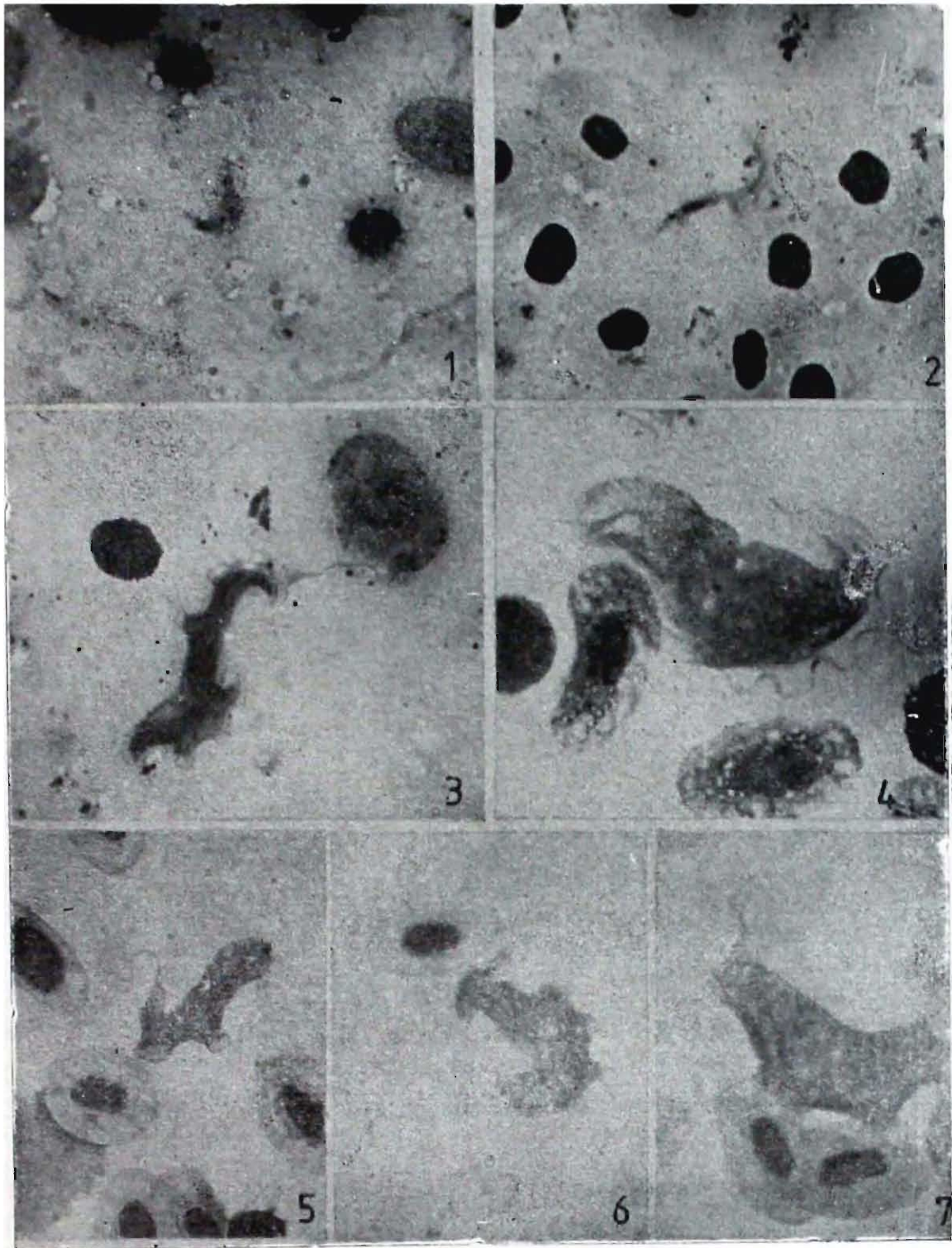


PLATE II

Figs. 1--8. Photomicrographs of *Trypanosoma rotatorium* from 4 anuran hosts.

1. Trypomastigote stage of *T. rotatorium* in *Bufo melanostictus* X 1500
2. The same in *Bufo stomaticus* X 1200
3. Typical leaf-like form of *T. rotatorium* in *Bufo stomaticus* X 1200
4. *T. rotatorium* (a dividing tryptomastigote) in *Rhacophorus malabaricus*, a tree frog. X 1230
5. Tryptomastigote stage of *T. rotatorium* in *Rhacophorus malabaricus*. X 1540
6. The same stage in *Rhacophorus maculatus* X 1450
7. An epimastigote stage of *T. rotatorium* in the kidney smear of *Rhacophorus maculatus* X 1375
8. A dividing amastigote stage of *T. rotatorium* showing 4 kinetoplasts and 4 nuclei. X 1000

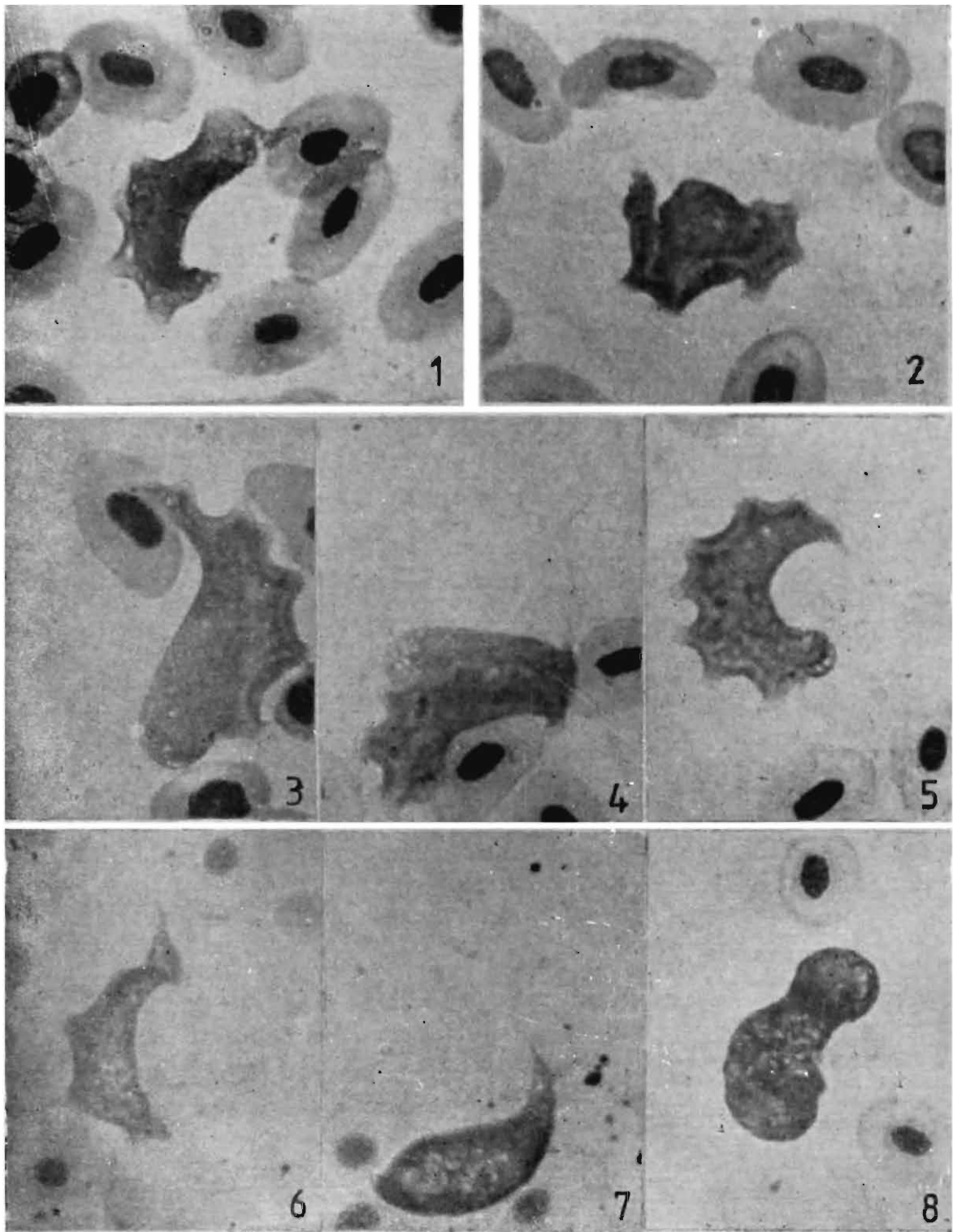


PLATE III

Figs. 1—8. Photomicrographs of the developmental stages of *Trypanosoma rotatorium* in a leech vector, *Helobdella nociva*.

1. Typical long-slender epimastigote form and two transitional form of *T. rotatorium* in the leech's gut X 1905 2. One metacyclic form and a long slender epimastigote stage of *T. rotatorium* X 1560 3. A dividing (unequal L. B. fission) epimastigote form X 1000 4. A typical longitudinal binary fission of *T. rotatorium*. Arrow indicates the line of cleavage. X 1425 5. A spheromastigote stage of *T. rotatorium* X 1265 6. An amastigote stage of the same X 1450 7. & 8. A number of stumpy short-membraned form in the crop smear of the leech, *Helobdella nociva*. X 1000

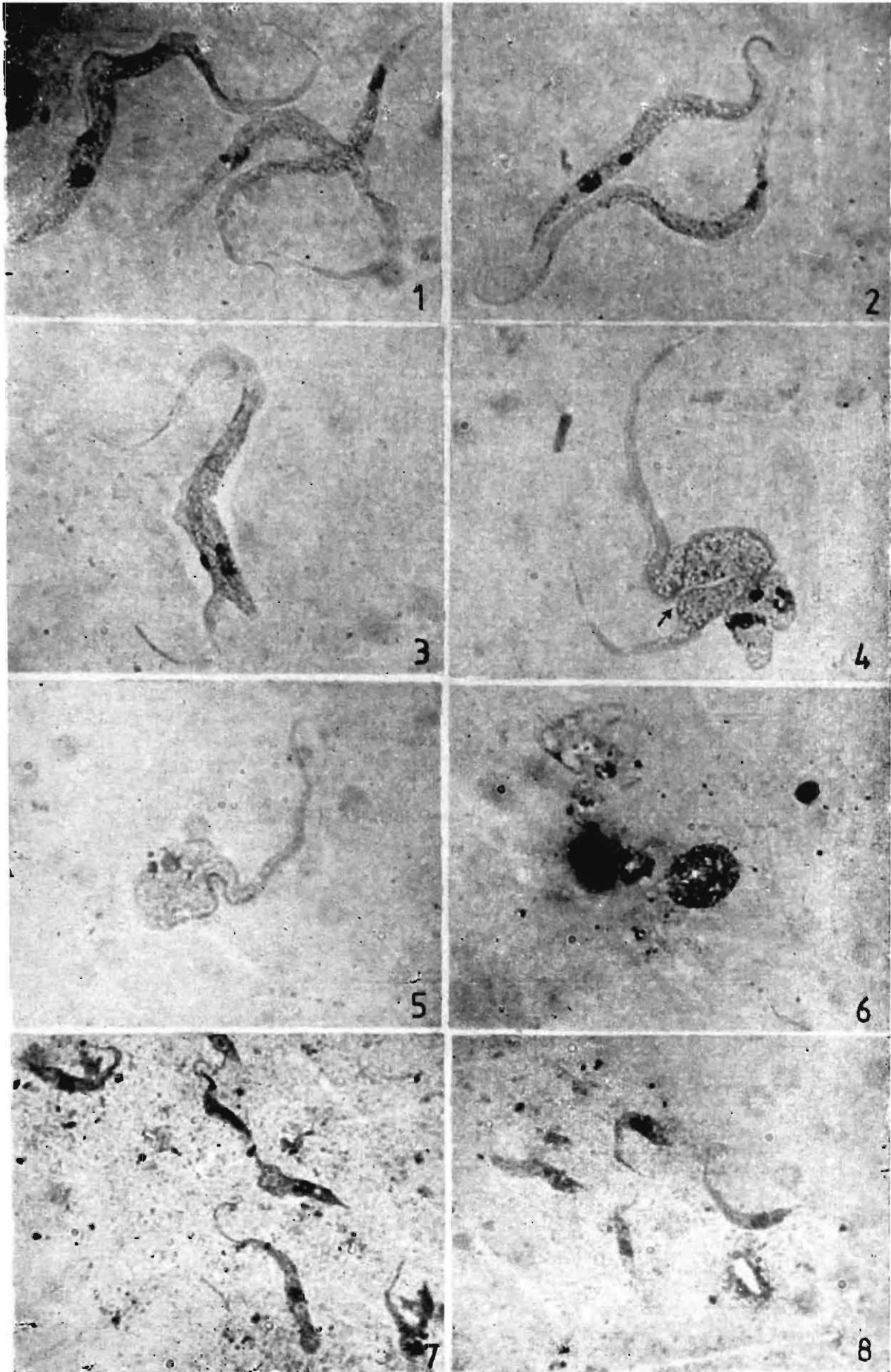


PLATE IV

Figs. 1—7. Photomicrographs of *Trypanosoma* spp.

1. Type I trypomastigote form of *Trypanosoma loricatum* in *Rana limnocharis* X 1000
2. Type II trypomastigote form of the same species in *Rana limnocharis* X 1100
3. *Trypanosoma karyozeukton* in *Rana hexadactyla* X 1500
4. *Trypanosoma chattoni* in *Rana limnocharis* X 1500
5. *T. chattoni* in *Rana cyanophlyctis* X 1570
6. *T. chattoni* in *Rana tigrina* X 1525
7. *T. chattoni* in *Bufo melanostictus* X 1460

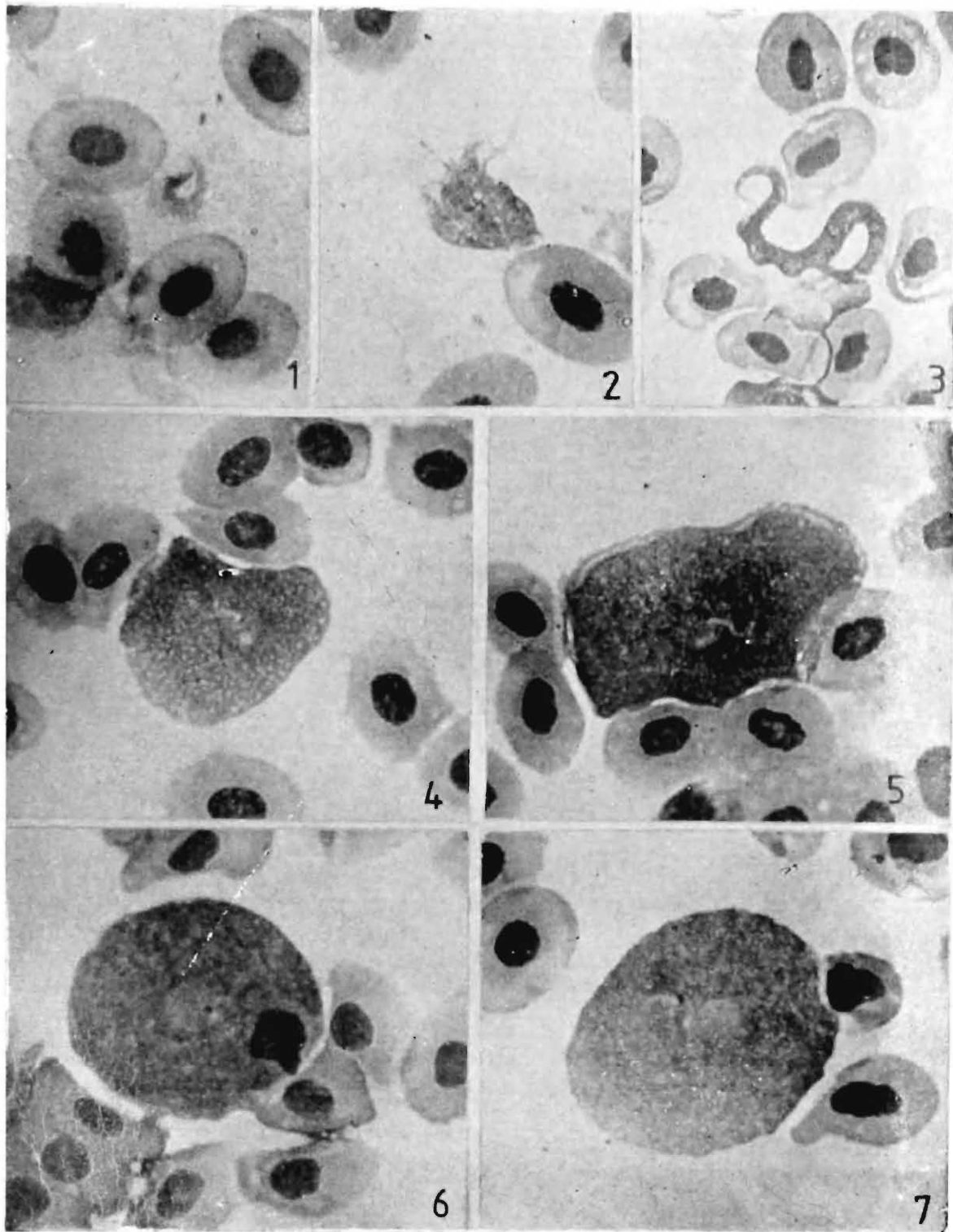
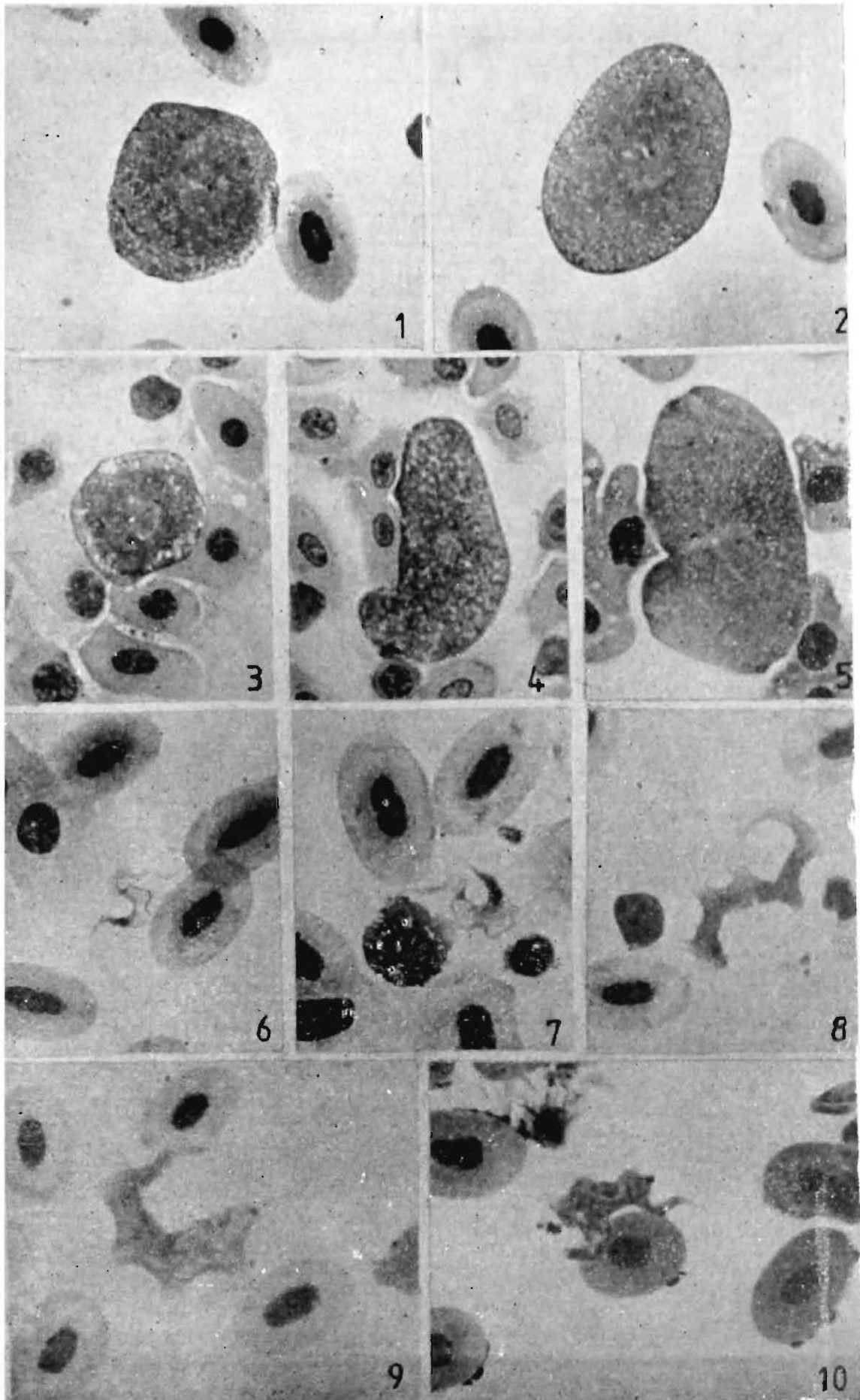


PLATE V

Figs. 1-10. Photomicrographs of *Trypanosoma* spp.

1. *Trypanosoma chattoni* in *Bufo stomaticus* X 1105
2. *T. chattoni* in the same host X 1250
3. *T. chattoni* in *Rhacophorus maculatus* X 1310
4. *T. chattoni* in *Rhacophorus malabaricus* X 1150
5. *T. chattoni* in *Microhyla ornata* X 1335
- 6 & 7. *Trypanosoma taprobanica* in *Kaloula pulchra taprobanica* X 1335, X 1500
- 8 & 9. *Trypanosoma malabarica* sp. nov. in *Rana malabarica* X 1500, X 1575
10. *Trypanosoma systoma* sp. nov. in *Uperodon systoma* X 1400



ERRATA

| Page | Line from top or bottom | For | Read |
|------|---------------------------|--------------------------------|---------------------------------|
| 1 | 2nd line | excellent | excellent |
| 2 | 3rd para 5th line | punctute | puncture |
| 3 | 3rd para 3rd line | measurement | measurements |
| 4 | 5th line from bottom | Pointed | pointed |
| 5 | 15th line | both the | both in the |
| .. | 20th line | eryxenuous | euryxenuous |
| 6 | 3rd line | Ledger | Larger |
| 7 | 7th line | Weyon | Wenyon |
| 9 | Last para 1st line | epimstigote | epimastigote forms |
| 13 | 3rd para 6th line | remained thoroughly | remained less thoroughly |
| 14 | 1st para 4th line | haemofflagellate | haemoflagellate |
| .. | 6th line | ecountered | encountered |
| 15 | Expl. of Fig. | FIG. A | FIG. II |
| .. | Last para 5th line | Noller (1912b) | Noller (1913b) |
| 17 | 11th line | <i>anarum</i> | <i>ranarum</i> |
| 19 | 10th line | (KI=4.79) | (KI=4.97) |
| 24 | 16th line | <i>Trypanosoma rotarium</i> | <i>Trypanosoma rotatorium</i> |
| .. | 17th line | <i>Cellulo</i> | <i>Cellule</i> |
| .. | 15th line from bottom | <i>Aychs</i> | <i>Archs</i> |
| .. | 3rd line | 30 | 33 |
| 27 | 4th line | Franka | Franca |
| 28 | 3rd line | Franea | Franca |
| 29 | 15th line | du sady | du sang |
| 30 | Fig. 13, drawing of | <i>Trypanosoma loricatum</i> | <i>Trypanosoma ranarum</i> |
| .. | Fig. 13 | Type II | Type II |
| .. | 3rd line from bottom | The vectos | The vector |
| 34 | 8th line | 23.5 | 23.5% |
| 35 | 15th line from bottom | authers | authors |
| .. | 16th line | characteristicts | characteristics |
| 37 | 3rd line | discerte | discrete |
| .. | 5th line | parasitie | parasitic |
| .. | 5th para 3rd line | <i>B. stomoticus</i> | <i>B. stomaticus</i> |
| 39 | 9th line | colon | colen |
| 45 | 12th line | RAY, R. and NANDI, NC. 1928 | RAY, R. and NANDI, N.C. 1978 |
| .. | 15th line | pt. 27-32 | pt. 3 : 27-32 |
| .. | 20th line | <i>rotato:ium</i> | <i>rotatorium</i> |
| .. | 23rd line | ROBARTSON | ROBERTSON |
| 47 | Table 1 PA | 13 83 | 13.83 |
| 48 | Table 2 12th line width | (7-10 5) | (7-10.5) |
| 49 | Table 4 3rd line | 0 36 | 0.36 |
| .. | .. 4th line | 0 54 | 0.54 |
| .. | .. 11th line | 0 21 | 0.21 |
| 50 | .. 5 3rd line from bottom | 0 02 | 0.02 |
| 52 | .. 9 1st line | <i>Rana hexadactyia</i> | <i>Rana hexadactyla</i> |
| .. | .. 5th line | (0.43-6.45) | (0.43-0.45) |