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BLOOD PARASITES OF INDIAN BULLFROG, *RANA TIGRINA* DAUD.

By

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(With 2 Plates and 2 Text-figures)

INTRODUCTION

In India, little attention has been paid to study the blood parasites of anuran amphibians. Berestneff (1903) reported a haemoflagellate, *Trypanosoma* from *Rana tigrina* for the first time from India. After that Patton (1908), Wenyon (1926), Scott (1926, 1927) and Pujati (1953) reported the occurrence of *Trypanosoma* and *Haemogregarina* in the blood of the same frog.

Recently during the survey on the blood parasites of Indian amphibians a total of 21 spp. of toads and frogs were examined (Ray and Mandal 1976, 1978 ; Ray 1977, 1979a b and Ray and Choudhury, 1980) of which *Rana tigrina*, a Bullfrog was found to be the most resourceful environment for a number of blood parasites and hence the present communication.

MATERIALS AND METHODS

The common Indian Bullfrog, *Rana tigrina* were collected from different districts of West Bengal and some were kept alive in the laboratory for study. Peripheral blood was obtained from the finger tips on alternate days and at autopsy. "Impression" and "Spread" preparations were made from liver, lungs, kidney and bone-marrow. Air-dried blood films and organ imprints were fixed in 100% methanol and stained with Romanowsky type of stains. For microtome section the tissue of lung, liver, kidney and spleen were fixed in Bouin's fixative, followed by general hitological technique by Pearse (1960), and stained with iron-haematoxylin and eosin.

Measurements were obtained from the camera-lucida drawings drawn on a graph paper (mm division) by counting the squares covered. The photomicrographs were taken with the help of "Ergavel" C. Z. microscope using PM6 attachment camera.

OBSERVATION

In course of investigation on the anuran haematozoa the author has examined 164 *Rana tigrina* of which 84 (51.21%) were found to be infected with various intracellular viz., *Haemogregarina magna* (17.6%), *H. perinucleophilum* sp. nov. (8.5%), *Lankesterella minima* (3%), *Pirhemocyton ranarum* (5.2%), *Cytamoeba bacterifera* (6.8%) and extracellular parasites viz., *Trypanosoma rotatorium* (31%), Microfilaria (0.6%). The brief descriptions of these parasites are as follows :

Trypanosoma rotatorium (Mayer, 1843)

(Pl. I, fig. 1)

1843. *Trypanosoma rotatorium* Mayer, 'Spicilegium observationum anatomicarum de organo electrico in *Ralis anelectricis* et de Haematozois ; Bonnae.

Description : *Trypanosoma rotatorium*, a pleomorphic haemoflagellate, is represented by 4 distinct forms viz., juvenile, slender, flat leaf-like and large compact form (Ray, 1979a, b). Amongst which the flat leaf-like forms are most predominant. They measure 29.0 μm in length and 4.5 μm in width. The free flagellum is very prominent and measures 24.0 μm in length. The cytoplasm, densely granular 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 2.4×1.6 μm, is situated on the posterior part of the body (NI=0.3).

Kinetoplast staining deep blue-black with Leishman, very small (0.8×0.8 μm) spherical or rod-like and surrounded by a halo, is situated at the extreme posterior end of the body (KI=2.5).

A few amastigote and epimastigote forms were detected in the blood and bone-marrow smears.

Remarks : The trypomastigote stage of *T. rotatorium* exhibited conspicuous pleomorphism with four distinct forms as reported by Wenyon, 1926, Mohammed and Mansour, 1959b. All these four forms of *T. rotatorium* have been found in the blood of *Rana tigrina* collected from a particular locale (Balitha, Bankura, West Bengal).

Haemogregarina magna (Grassi and Feletti, 1891)

(Pl. I, figs. 2-8 & Text-figs. 1 a-j)

1891. *Haemogregarina magna* Grassi and Feletti, *Cert. f. Bakt. u. Parasit.* **10** : 449.

Description : *Intra-cellular gametocytes* (Pl. I, fig. 2 & Text-fig. 1 a, b) : Gamonts are elongated with broadly rounded anterior end and a little

They measure 1.9×1.05 μm and 1.3 μm^2 in area (Pl. I, fig. 4 & Text-fig. 1 j).

Schizonts were also found in the endothelial cells or free in the capillaries of the lung tissue. The immature uninucleate schizonts are spherical lay in a parasitophorous vacuole, measuring 5.00-10.00 μm×4.00-9.5 μm. The mature schizonts are spherical to subspherical, measuring 19.5-42.0×19.0-43.0 μm with an average of 31.57 μm and contain 30 or more merozoites. (Pl. I, fig. 8). They are elongated lanceolate, straight or curved bodies measuring 5×1.3 μm.

Locality : Bishnupur, Bankura district, West Bengal.

Remarks : Berestneff (1903), Patton (1908), and Wenyon (1926) recorded this parasite from Indian frog, *Rana tigrina*. Recently Levine and Nye (1977) observed this parasite from the frogs of Illinois, New York, Tennessee and Wisconsin and substantiate the species description with quantitative measurements : Morphometric measurements of the parasite obtained from *Rana tigrina* from West Bengal, India, are included herein and the erythrocytic schizogony of the parasite is described for the first time.

Haemogregarina perinucleophilum sp. nov.

(Pl. II, figs. 1 & 2 and Text-figs. 2 a-j)

Type host : *Rana tigrina* Daudin.

Type locality : Balitha, Bankura District, West Bengal.

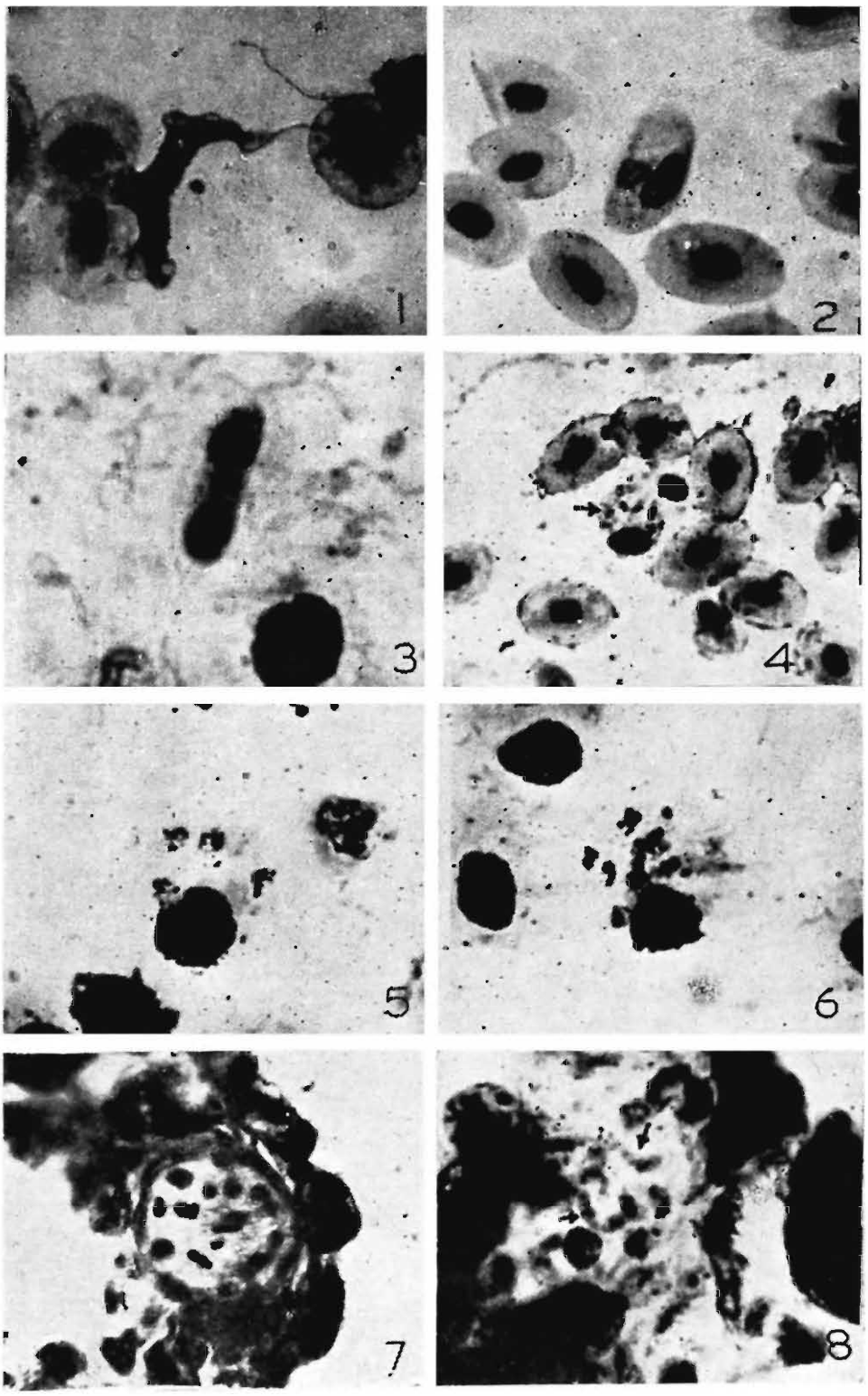
Registration No. *Holotype* Pt. 1953.

Paratype Pt. 1954.

Description : *Young and Growing forms* (Text-fig. 2 e-h) : There are two forms—broad and thin form. The broad forms are elongated with both the ends rounded, occur mostly within the immature erythrocytes. They measure 9.0×3.2 μm and 22.8 μm^2 in area. Cytoplasm is densely granular. Nucleus is composed of chromatin rods at the centre of the parasite crossing the entire width of the body. It measures 3.5×2.0 μm and 6.5 μm^2 in area.

The thin forms are also elongated, measuring 8.0×1.5 μm and 13.25 μm^2 in area. The cytoplasm is densely granular, stained dark blue. Nucleus measures 4×2 μm and 4.5 μm^2 in area, stained purple red with Wright and Giemsa stains.

Elongate mature form : Mature parasites are elongated gregariniform having a broader head, attenuated thin body and a hook-like pointed tail. The parasite always encircles the host cell nucleus by its twisted



Figs. 1-8. 1. Typical leaf-like form of *Trypanosoma rotatorium* × 1100

2. Intra-cellular gametocyte of *Haemogregarina magna* × 1250

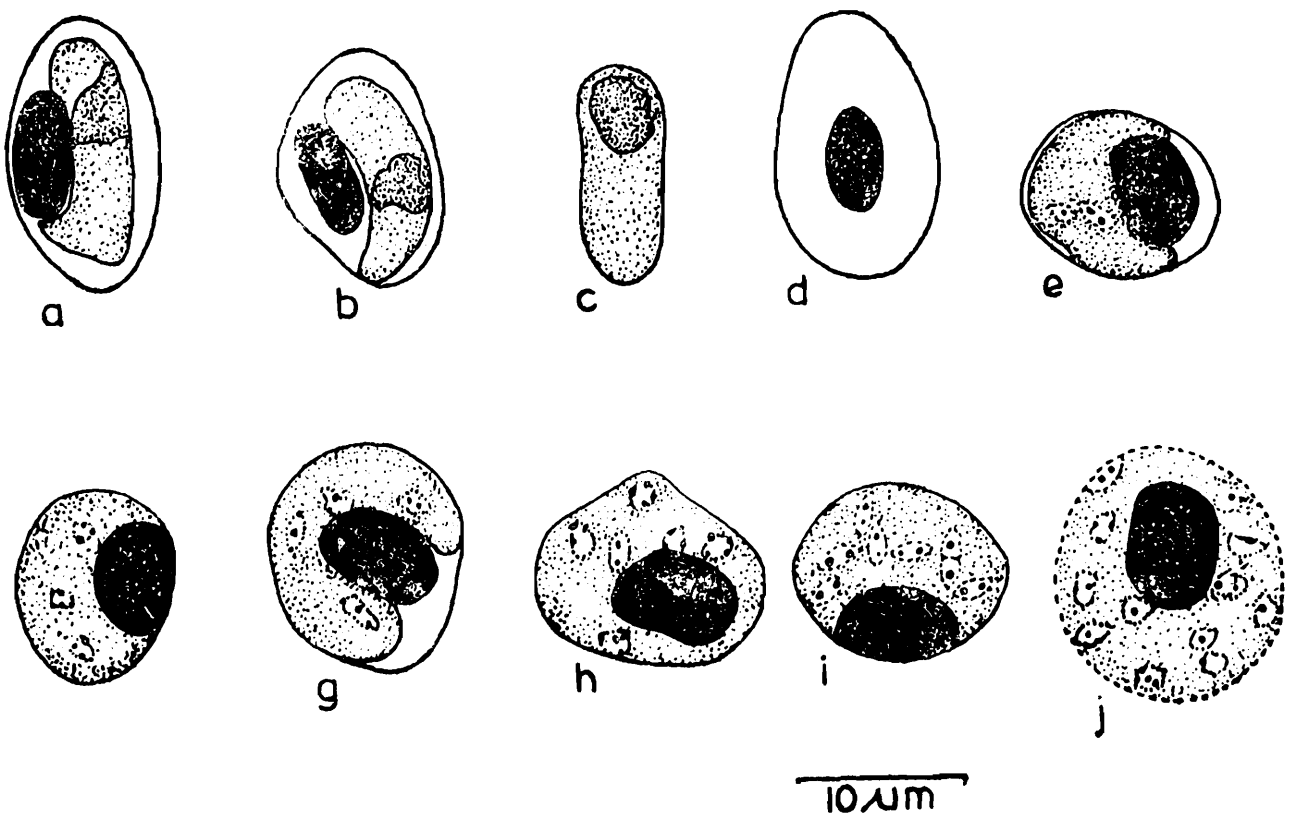
3. Free gametocyte of *H. magna* × 1335

4-6. Erythrocytic schizonts (arrow) of *H. magna* × 1000, × 1250, × 1250

7. A mature schizont in the endothelial cells of lung capillaries × 1760

8. The merozoites (arrow) in the lung tissue × 1000

narrower posterior end. The mature form is 12.4×4.06 μm and 44.5μm^2 in area, occupying about 42.7% of the total host cell-parasite complex. Cytoplasm homogeneous, hyaline, a little granular and stained pinkish blue with Giemsa stains. The nucleus is broad to oval, sometimes rounded or band-shaped. It measures 4.4×3.3 μm and 12.1 μm^2 in area. The parasite was never found to be enclosed in a capsule.

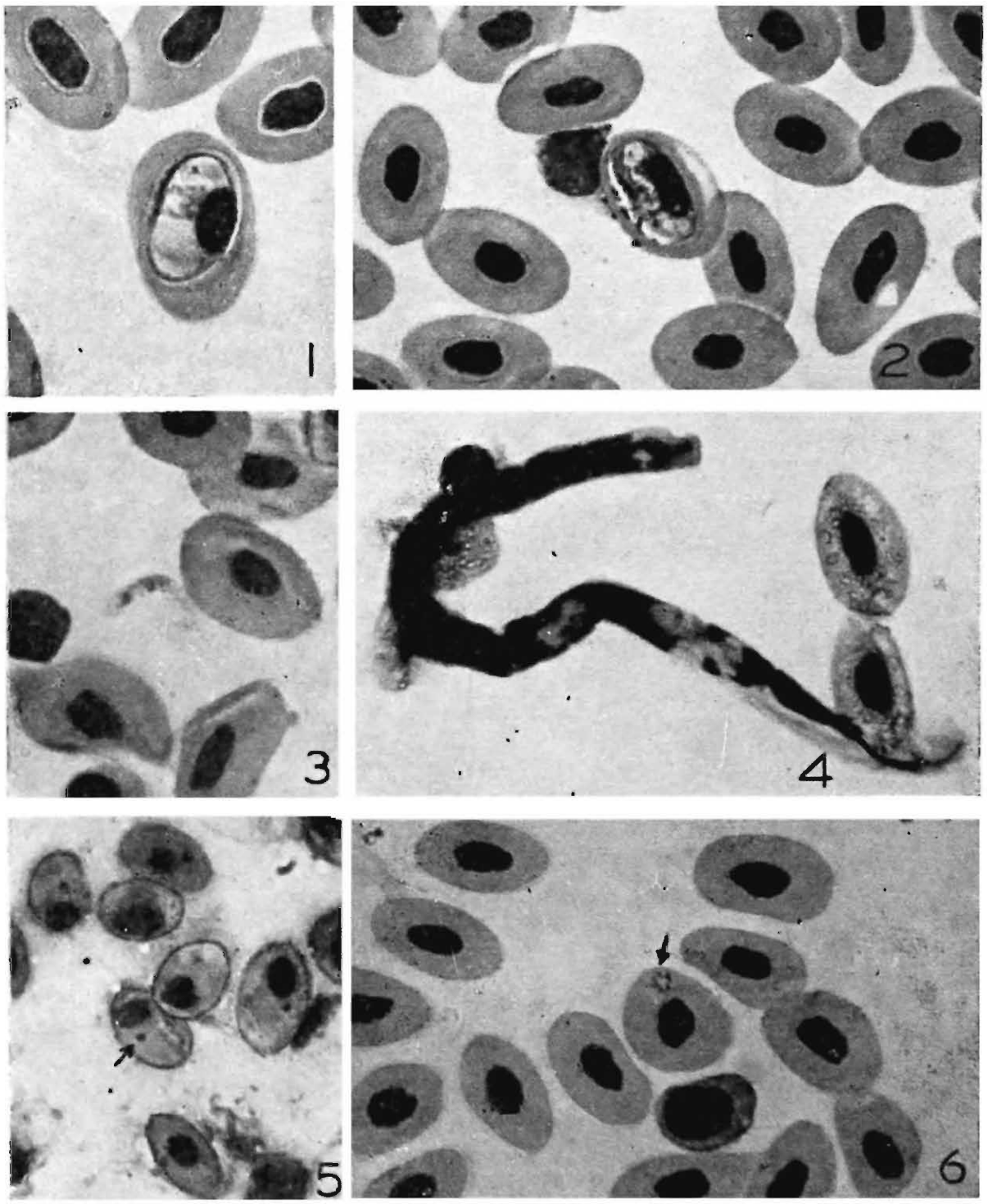


Text-fig. 1 a-j. Camera-lucida drawings of the erythrocytic stages of *Haemogregarina magna* from *Rana tigrina*. a & b, the mature macrogametocytes ; c, the extracorpuscular free gametocytes ; d, the uninfected erythrocytes. ; e, the bi-nucleated schizont ; f, the tri-nucleated schizont ; g, the quadri-nucleated schizont ; i, the mature schizont with developing merozoites ; j, the mature erythrocytic schizont with 12 nuclei.

Free gametocyte (Pl. I, fig. 3 & Text-fig. 1 c) : In some cases free forms were seen in the blood plasma. They are simillar in appearence like that of intracellular gametocytes. They measure 11.4×4.16 μm and 47.7 μm^2 in area. The nucleus is compact, rounded measuring 4.36× 3.25 μm and 12.21 μm^2 in area, and always situated towards the anterior side.

Schizogony : Two types of schizogony both in the R. B. C. and in the endothelial cells of the lung tissue were observed. (Pl. I, figs. 4-8 & Text-fig. 1 e-j).

Erythrocytic schizont measures 12.5×10.70 μm and 103.9 μm^2 in area. Mostly bi-, tri- and tetranucleated schizonts were found. The mature schizont possesses 12 fully developed spindle shaped merozoites.



Figs. 1-6. 1. Microgametocyte of *Haemogregarina perinucleophilum* sp. nov. × 3550

2. Macrogametocyte of *H. perinucleophilum* sp. nov. × 2165

3. Free sporozoite of *Lankesterella minima* × 1750

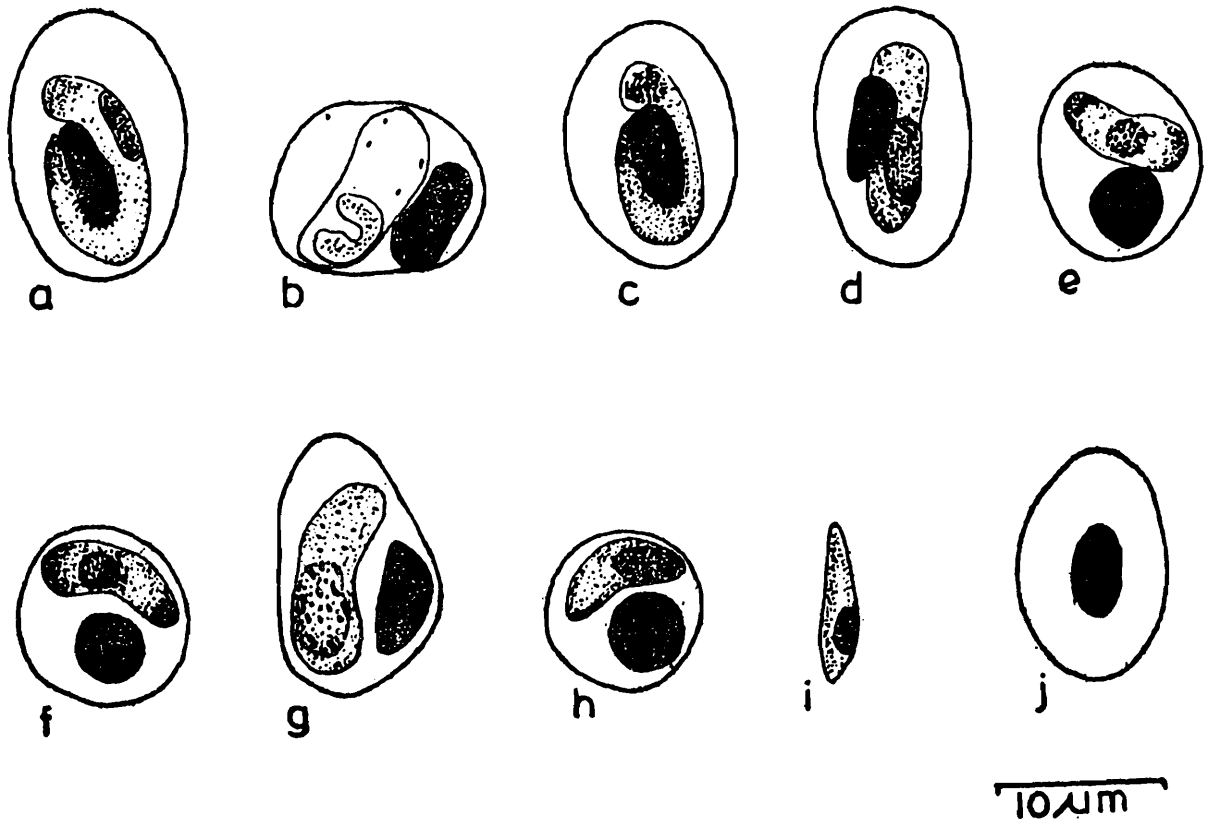
4. Microfilaria in the blood of *Rana tigrina* × 1000

5. *Pirhemocyton ranarum* (arrow) in the blood of same frog × 1250

6. *Cytamoeba bacterifera* (arrow) in the same host × 1250

body. In some cases they may push the host cell nucleus at one corner. They measure 11.1×3.1 μm and 25.7 μm^2 in area. One parasite measures 18.5×4.0 μm with an area of 37.5 μm^2, occupying about 29.3% of the total host cell-parasite complex.

Cytoplasm densely granular, thicker at the ends ; stained deep blue with Leishman's stain. Nucleus is oval or kidney shaped, situated at the anterior half of the body. It measures 3.4×2.2 μm with an area of 6.0 μm^2 and contains less compact chromatin granules. The capsule is not present in this parasite. The microgametocyte possesses a very clear cytoplasm and less compact, band-like nucleus. Macrogametocyte has dense cytoplasm and a compact nucleus (Pl. II, fig. 2).



Text-fig. 2 a-j. Camera-lucida drawings of the erythrocytic stages of *Haemogregarina perinucleophilum* sp. nov. in *Rana tigrina*. a, the macrogametocyte ; b, the microgametocyte ; c & d, the mature macrogametocytes ; e-g, the young broad forms of gametocytes ; h, the young thin forms ; i, the extracorpuscular free form ; j, the uninfected erythrocyte.

Extra-corpuscular free form (Text-fig. 2 i) : Small, elongated, gregariniform, comparatively fewer in number. Anterior end broader than the posterior which is more or less pointed. They measure 9.6×2.6 μm and 13.9 μm^2 in area. Cytoplasm homogeneous, granular ; stained deep blue with Leishman. The nucleus is rounded or ovoid, sometimes elongated, situated at the anterior half of the body, towards the broader end. It measures 2.7×1.5 μm and 2.0 μm^2 in area.

Effect of parasite on the host cell : The infected erythrocytes became hypertrophied with the displacement of host cell nucleus even in early infection. The host cell nucleus became rounded and sometimes pushed to one corner of the host cell. Host cell hypertrophied only in width (12.79%). Host cell nucleus hypertrophied in area (26.22%).

Occurrence : Out of 164 examples of *Rana tigrina* examined 14 were found to be infected with this parasite.

Remarks : A perusal of the literature revealed that only two species of haemogregarine *viz.*, *Haemogregarina berestneffi* Castellani and Willey and *H. magna* (Grassi and Feletti) were recorded so far in the host *Rana tigrina* from India. Patton (1908) had the opportunity of studying a good number of *Haemogregarina* from amphibian hosts and suggested the presence of some different haemogregarines within the same host.

Haemogregarina perinucleophilum sp. nov. is distinguished from the other haemogregarines by the fact that the mature gametocytes characteristically encircles the host cell-nucleus with its twisted body after which the specific name of the parasite has been coined. The present parasite differs from *H. berestneffi* in not having the capsule and also on the basis of morphometric measurements (Table 1). It can also be differentiated from *H. magna* by its characteristic encircling nature of the parasite to its host cell nucleus and by the position and structure of the parasite nucleus.

Lankesterella minima (Chaussat, 1850)

(Pl. II, fig. 3)

1850. *Lankesterella minima* Chaussat, 'Des hematozoaires (Thesis for the Doctorate degree in Medicine, Paris).

Description : *Sporozoite* : The vermiform elongated sporozoite measure 10.0-11.5 μm in length with a mean of 10.5 μm and 1.0-2.0 μm in width with a mean of 1.5 μm. The nucleus is median to subterminal with a clear refractile area towards the periphery. The cytoplasm is homogeneous, hyaline with more darker margins and stained faint blue. The delicate nucleus is of vesicular type with a central irregular karyosome and peripheral coarser chromatin granules of pinkish colour with Wright stains. Two vacuoles are very clear, located anterior and posterior to the nucleus. An extranuclear granule is also visible at the anterior end of the sporozoite.

After invading the erythrocytes the sporozoites form a crescent-shaped area within the erythrocytes. The intracorpuscular sporozoites became shorter and wider, sometimes attain a greater width.

TABLE 1 : Comparative morphological measurements of the gametocytes of *H. berestneffi*, *H. magna* and *H. perinucleophilum* sp. nov. recorded from *Rana limnocharis* and *R. tigrina* in the present study. Linear measurements in micrometers and area measurements in micrometer^2. Standard deviation in parenthesis.

	H. berestneffi	*H. magna*	*H. perinucleophilum.*
Uninfected RBC			
Length	12.9(0.37)	15.6(1.26)	13.0(1.5)
Width	9.1(0.53)	8.5(0.52)	8.6(0.82)
Area	86.97(9.41)	94.4(12.95)	87.0(11.25)
Nuclear area	13.04(2.13)	12.2(1.92)	11.10(2.25)
NDR*	0.95(0.05)	0.92(0.06)	0.90(0.07)
Infected RBC			
Length	14.05(0.43)	15.1(0.83)	12.8(2.18)
Width	10.50(0.83)	9.3(0.41)	9.7(1.05)
Area	101.45(12.04)	104.2(8.02)	87.7(16.42)
Nuclear area	13.97(2.12)	18.3(2.81)	13.2(2.84)
NDR*	0.23(0.08)	0.22(0.15)	0.26(0.21)
Intracorpuscular parasite (Gametocyte)			
Length	16.50(0.76)	12.4(1.11)	11.1(3.3)
Width	2.57(0.32)	4.06(0.23)	3.1(0.76)
Area	29.56(3.18)	44.50(7.09)	25.7(7.06)
Nuclear area	5.13(0.76)	12.10(1.93)	6.0(1.90)
Extracorpuscular parasite (Free gametocyte)			
Length	17.60(0.74)	11.40(1.68)	9.6(0.37)
Width	2.30(0.22)	4.16(0.23)	2.6(0.37)
Area	27.70(1.28)	47.70(6.81)	13.9(0.95)
Nuclear area	4.77(1.34)	12.21(2.05)	2.0(0.26)

* NDR = Nuclear Displacement Ratio.

Developmental stages : The sporozoites make their way into the blood capillaries of various organs and enter the endothelial cells where the entire development takes place. The uninucleate schizonts are spherical measuring 7.5-8.5 μm in diameter. Tri-or pentanucleated schizonts are also being encountered in the tissue preperation of lungs and liver. The schizonts fully packed with merozoites numbering 16-25 are also found in the section of lungs.

The merozoites are elongated with one end tapering than the other. They measure 6.0 μm in length and 1.5 μm in width. The spherical macro and microgametocytes measure 6-8 μm. The oocysts are spherical containing 10-20 sporozoites and a compact oocystic residual mass.

Locality : Bankura and 24-Parganas districts, West Bengal.

Remarks : Ray and Mandal (1978) described the life-cycle of *Lankesterella minima* in detail. Previously Bhatia (1938) in his monumental work on the Fauna of British India, mentioned the occurrence of *Lankesterella minima* from *Rana tigrina* and presented the life-cycle based on Nöller (1912, 1920). The findings of the present author well corroborated with Nöller's findings.

Dactylosoma Labbe'

1894. *Dactylosoma* Labbe', *Arch. Zool. exp. gen.*, **2** : 54.

Generic diagnosis : The genus *Dactylosoma*, Labbé, 1894, parasitising the erythrocytes of amphibia, is characterized by the presence of schizogony ; nucleus with an endosome, a less granular and more vacuolated cytoplasm at all stages. It lacks pigment granules. The schizonts of *Dactylosoma* produce 4-16 merozoites, often with the predivision nuclei arranged somewhat quadrilateral mass (Manwell, 1964).

Remarks : Manwell (1964) has made an up-to-date survey of the literature of this parasite. A large number of species of *Dactylosoma* have been described from fishes, frogs and toads as well as from reptiles.

This parasite though wide spread in its occurrence both in frogs and some other vertebrates, it could not be observed in any of the hosts examined during the present study and till now species of *Dactylosoma* from Amphibians has not been reported from India.

Larval Nematode (Microfilaria)

(Pl. II, fig. 4)

Description : The microfilariae are long, slender, serpent-like measuring 92.2-100.5 μm in length and 4.5-5.0 μm in width. Cephalic space is more or less rounded measuring 2.5 μm in diameter. The nuclear column consists of many nuclei haphazardly arranged and stained deep red with Leishman. There are four oval shaped 'G' cells. The first 'G' cell is situated a little behind the second. The tail is 13.0 μm in length which terminates gradually into a pointed end. A spoon shaped prolongation of the sheath is well marked beyond the tail which measures 24.5 μm in length and stained reddish in colour. Position of land marks : nerve ring 22.5-24.4% ; excretory pore 39.5-42.8% ; central viscous 67.7-68.0% ; anus 68.5-74.2%.

Locality : Balitha, Bankura District, West Bengal.

Remarks : Walton (1964) listed some larval nematodes (microfilariae) from frogs. Mathis and Léger (1911) (cf Walton A. C., 1950) reported

Microfilaria sp. from Indo-China region. Nandi and Mandal (1977) reported the occurrence of microfilariae from *Bufo melanostictus* in India. Present author observed sheathed microfilaria from the peripheral blood of *Rana tigrina* which constitutes the second host record of its kind and first report from India in this host.

Doubtful Protozoans

Pirhemocyton ranarum Ray and Mandal

(Pl. II, fig. 5)

1976. *Pirhemocyton ranarum* Ray and Mandal, *Proc. zool. Soc. Calcutta*, **31** : 85.

Description : The parasites are small 1-2 μm in diameter with a central chromatin dot surrounded by clear cytoplasm which stained pink with Wright stain. Sometimes the chromatin dots are located at the periphery. In some cases the chromatin dots appeared to be dividing into 2 or 3. The parasites are usually in contact with an unstained globular body.

Locality : Tarakeshwar, Hooghly District, West Bengal.

Remarks : Chatton and Blanc (1914) observed this organism in the red blood cells of North African Gecko and established the genus *Pirhemocyton*. Dasgupta (1959, 1961) did some cytochemical observations on *Pirhemocyton* of *Rana occipitalis*. Recently Ray and Mandal (1976) described this species of *Pirhemocyton* from an Indian Bullfrog *Rana tigrina*. The taxonomic position of this organism is doubtful even today.

Cytamoeba bacterifera Labbé

(Pl. II, fig. 6)

1894. *Cytamoeba bacterifera* Labbe', *Arch. Zool. exp. gen.*, **2** : 58.

Description : The parasites are of two morphological types. Smaller types are circular measuring 3-4 μm in diameter, intra-cellular, contained in a clear space within the R. B. C. The parasites stained light blue with Leishman's stain and having small dot-like dark granules scattered throughout the cytoplasm.

The larger types are also circular, measuring 5.0-6.5 μm in diameter, situated at the periphery of the erythrocytes. Other characters are similar like the small forms.

Locality : Sagar Islands, 24-Parganas, West Bengal.

Remarks : *Cytamoeba bacterifera* has been observed by a number of workers in the blood of anuran amphibians. Kruse (1890), probably

the first to figure this parasite, speaks of it not as an organism but as a space in the blood. Labbé (1894), Ziemann (1898), Laveran (1899) Dutton *et al.* (1907), Lehmann (1961) reported this organism from anuran amphibians. Ray (1979 b), observe this organism from anuran amphibians of India. The taxonomic status of this parasite is also doubtful to-day even though they may be listed in the protozoology textbooks.

Discussion

In course of investigation on Amphibian haematozoa a number of blood parasites were found to infect the common Indian bullfrog *Rana tirgina*. The blood parasites which are recorded from this frog include the genera *Trypanosoma*, *Haemogregarina* and *Lankesterella* belonging to phylum protozoa and larval nematodes like microfilaria of the phylum Aschelminthes. Besides these some intracellular organisms viz. *Cytamoeba* and *Pirhemocyton* were also recorded whose taxonomic position is a matter of dispute even today.

Trypanosoma rotatorium is pleomorphic and shows 4 distinct trypomastigote forms. A considerable number of haemogregarine species have been recorded from amphibians, but observation on schizogonic development has been so seldom made. Mohammed and Mansour (1959a) opined that the shape, cytoplasmic and nuclear structure, staining reactions, the effect of the gametocytes on the host cells and nuclei may be accounted as reliable criteria.

Howerver, current diagnosis of the genus *Haemogregarina* (*sensu strictu*) may specify the occurrence of schizogony in the cells of the circulating blood. The generic name *Haemogregarina* may be used in its broad sense (*sensu latu*) for those species in which schizogony in the circulating blood is unknown. The author proposes the generic term *Haemogregarina* (*sensu strictu*) for *Haemogregarina magna* which exhibits typical schizogony in the circulating blood of *Rana tigrina* and *Haemogregarina* (*sensu latu*) for *H. perinucleophilum* sp. nov. in which case schizogony could not be observed. Nöller (1912, 1920) is credited for his pioneer contribution to the study of the life-cycle of *Lankesterella minima* and his experiments with leech as mechanical vector. Nöller's work on the life-cycle of *L. minima* is endorsed with morphometric measurements.

A perusal of the literature revealed that there is only one report of microfilaria from *Rana tigrina* (Walton, 1950) where Mathis and Léger (1911) reported the same from Indo-China region. The microfilaria reported herein constitutes the second host record of its kind and first report from India in this host. Ray and Mandal (1976) described

Pirhemocyton ranarum from *Rana tigrina* for the first time in India. ***Cytamoeba*** is also reported from this frog for the first time from India.

SUMMARY

1. The paper deals with the description of some intracellular and extracellular parasites of common Indian edible frogs, *Rana tigrina* Daud.
2. The extracellular protozoan parasite *Trypanosoma rotatorium* shows pleomorphism in the host *Rana tigrina* collected from a particular locality.
3. *Haemogregarina magna*, an intracellular blood parasite has been reported herein along with its erythrocytic schizogony and tissue schizogony for the first time in India.
4. *Haemogregarina perinucleophilum* sp. nov. has been described as new to science ; *Lankesterlla minima*, a haemococcidia has reported alongwith morphometric measurements.
5. A Microfilaria and some doubtful organism like *Pirhemocyton* and *Cytamoeba* have also been described in the present paper.
6. The current diagnosis of the genus *Haemogregarina* has been discussed.

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