

## BLOOD PARASITES OF INDIAN TOADS

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(With 2 Figures and 2 Plates)

### INTRODUCTION

Though the amphibians are known to host a large number of parasites belonging to different phyla, little attention was given to study the haematozoan parasites of amphibians. During the early nineteenth century some stray reports are available viz., Shortt (1917) and Wenyon (1926) who worked on the trypanosomes and haemogregarines of Indian toads. Recently Ray (1979a, b, 1980, 1982), Tiwari & Ray (1981) and Sinha (1979) published some papers on the blood parasites of Indian toads. No consolidated work has even been undertaken on this particular aspect. In this context, a brief account on the blood parasites known so far from the Indian toads is being dealt with in the present communication.

### MATERIAL AND METHODS

Four hundred and seventy eight examples of toads comprising of 4 species viz., *Bufo melanostictus*, *B. andersoni*, *B. stomaticus* and *B. himalayanus* were collected from different parts of India and examined for blood parasites. 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 histological techniques by Pearse (1960), and stained with iron-hematoxylin and eosin.

Measurements were obtained from the camera-lucida drawing drawn on a graph paper (mm division) by counting the squares covered. The

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

#### OBSERVATIONS

During the study on anuran haematozoa the author observed 5 intracellular parasites viz., *Haemogregarina nucleobisecans*, *Lankesterella bufonis*, *Pirhemocytion* sp., *Cytamoeba bacterifera*, *Toddia* sp. and 3 extracellular parasites viz., *Trypanosoma rotatorium*, *T. chattoni* and *Microfilaria* which have been enumerated in Table—I.

Among the parasites dealt with so far *Pirhemocytion* sp. and a sheathed microfilaria are recorded for the first time from Indian toads. Moreover,

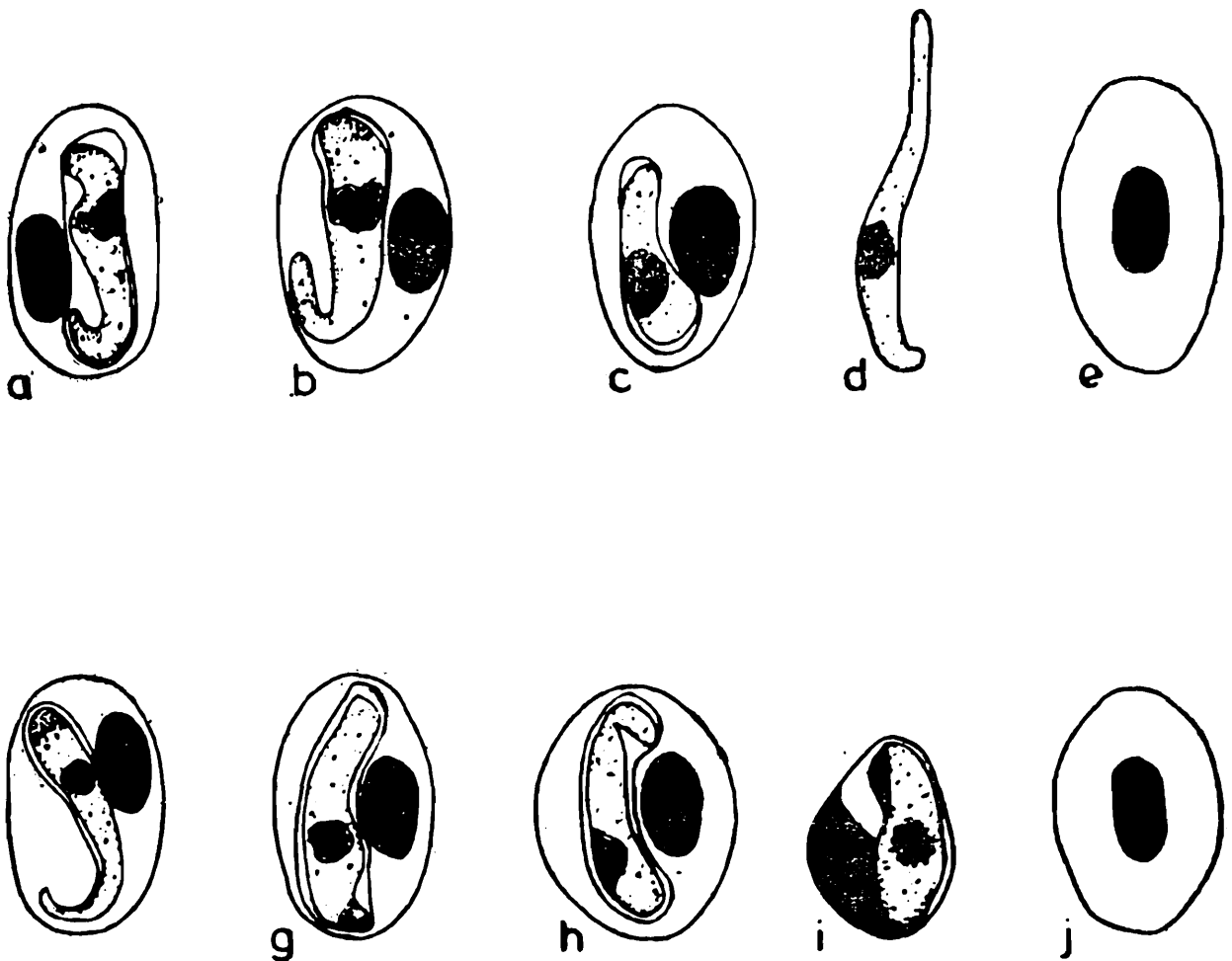


FIG. 1. HAEMOGREGARINA NUCLEOBISECANS.

10µm

Fig. 1. Camera-lucida drawings of the erythrocytic stages of *Haemogregarina nucleobisecans* from two anuran hosts.

a-d, the gametocyte stages in *Bufo melanostictus*: a & b, the longate mature form; c, the young form; d, the free gametocyte; e; the uninfected erythrocyte.

f-i, the gametocyte stages in *Bufo andersoni*; j, the uninfected erythrocyte.

some interesting observations on those parasites have also been made. In view of this those are discussed below :

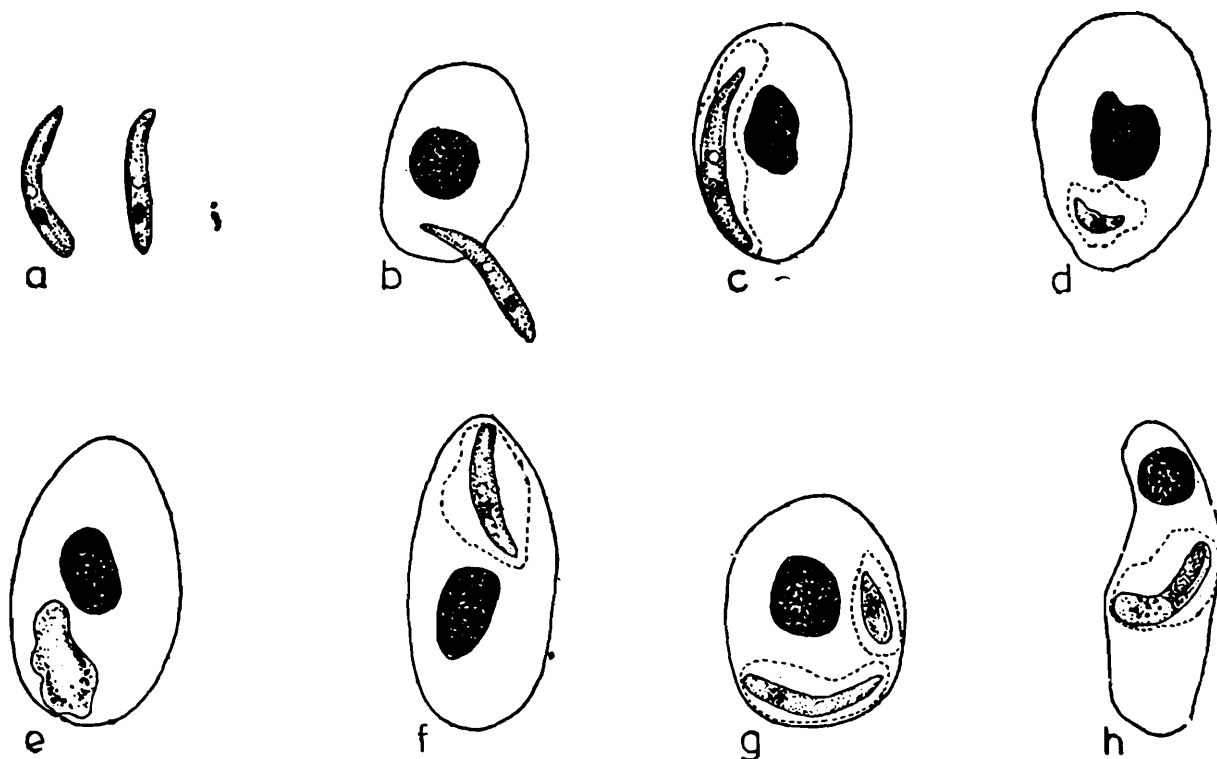


FIG.2 . LANKESTERELLA BUFONIS.

10  $\mu$ m

Fig. 2. Camera-lucida drawings of *Lankesterella bufonis* from *Bufo melanostictus*.

a, the extracorporeal sporozoites ; b, the sporozoite invading the erythrocyte ; c-h, the intracorporeal sporozoites ; g, the hypertrophied cell with double infection ; h, the infected R. B. C. became elongate with apical displacement of the cell nucleus.

### **Pirhemocyon sp.**

(Plate IV, Figs. 10, 11)

*Hosts* : *Bufo melanostictus* & *B. himalayanus*.

*Description* : These are intracorporeal small rounded bodies measuring 1.5-2.0  $\mu$ m in diameter. There is a round, central chromatin dot surrounded by thin layer of cytoplasm which sometimes becomes non-visible because the enlarged chromatin material covers the body. There may be 2-3 small associated unstained refractile globules but not always present. These globules when present, are 1-2  $\mu$ m in diameter. There is no remarkable change in the infected erythrocytes.

*Locality* : Balitha, Bankura district and Darjeeling, West Bengal.

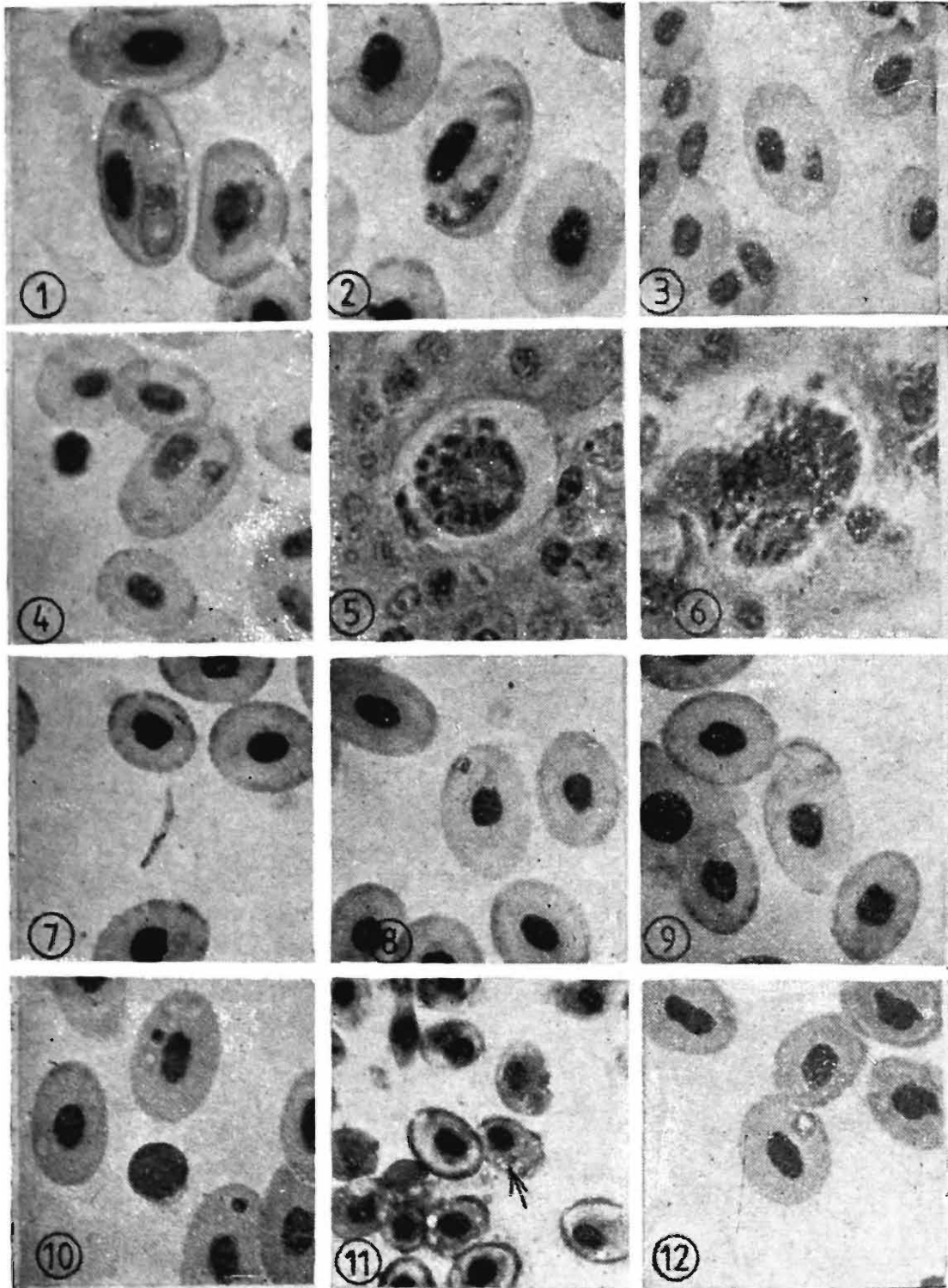
*Occurrence* : 12, out of 343 examples of *B. melanostictus* and 4 out of 32 *B. himalayanus* were positive for *Pirhemocyon*.

TABLE—1

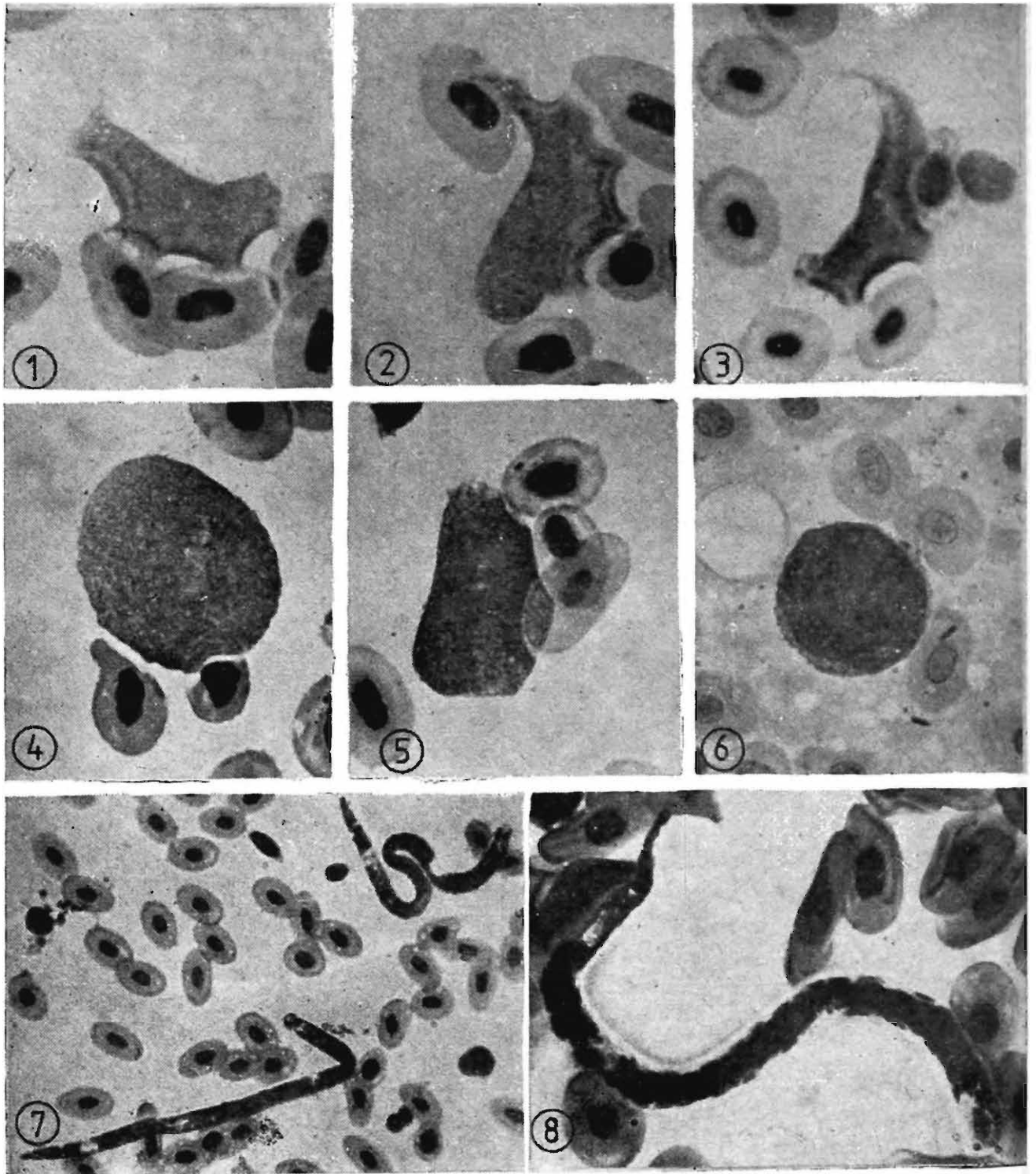
Parasite	Name of the hosts	Locality	Diagnostic characters	Remarks	References
1. <i>Haemogregarina nucleobisecans</i> (Text-figs. 1a-j ; Pl.1, figs. 1-6)	<i>Bufo melanostictus</i> and <i>B. andersoni</i>	Bankura & Purulia District West Bengal, India.	Sausage-shaped endoglobular parasites of erythrocytes ; gametocytes measuring $14.4 \times 2.95 \mu\text{m}$ with an average area of $28.95 \mu\text{m}^2$ ; schizogony in the hepatic cells of the liver which produces merozoites.	This species has also been recorded from a new host, <i>Bufo andersoni</i> .	Short t (1917), Wen yon (1926), Ray (1979b,1982).
2. <i>Lankesterella bufonis</i> (Text-figs. 2a-g ; Pl. 1, figs. 7-9)	<i>Bufo melanostictus</i>	Balitha and Kotalpur, Bankura District, West Bengal, India.	Very small, slender, crescent-shaped parasites measuring 9-11.5 $\mu\text{m}$ in length and 1.0-2.5 $\mu\text{m}$ in breadth with an average of $10.6 \mu\text{m} \times 1.45 \mu\text{m}$ ; nucleus terminal or subterminal and consists of few chromatin granules ; a single vacuole situated centrally.	The report of this parasite represents a new host-parasite record from a new geographical region (Oriental realm).	Mansour & Moham med (1962), Ray (1979b)
3. <i>Pirhemocytton</i> sp. (Pl. 1, figs. 10, 11)	<i>Bufo melanostictus</i> & <i>B. himalayanus</i>	Balitha, Bankura District and Darjeeling, West Bengal, India.	Intracellular, small rounded bodies measuring 1.5-2.0 $\mu\text{m}$ in diameter ; central chromatin dot surrounded by thin layer of cytoplasm ; 2-3 small associated refractile globules may be present.	This parasite has been recorded from two new hosts viz. <i>B. melanostictus</i> and <i>B. himalayanus</i> .	Ray (1979b)
4. <i>Cytamoeba bacterifera</i> (Pl. 1, fig. 12)	<i>Bufo melanostictus</i> & <i>B. himalayanus</i>	Balitha, Bankura Districts and Darjeeling, West Bengal, India.	Small (3-5 $\mu\text{m}$ in diam.), amoeboid or spherical, intracorpular red staining bodies ; cytoplasm homogeneous or granular and contains crystalline bacillus-like bodies.	This parasite has been recorded for the first time from Indian toads.	Labbé (1894), Ray (1979b).
5. <i>Toddia</i> sp.	<i>Bufo melanostictus</i> and <i>Bufo andersoni</i>	Burdwan & Purulia, West Bengal, India.	Red staining spherical structure (1-3 $\mu\text{m}$ in diameter) ; intracellular ; rods of a crystalline nature associated with the parasite.	This parasite has been recorded for the first time from the toad of West Bengal.	—

TABLE—1 (Concluded)

Parasite	Name of the hosts	Locality	Diagnostic characters	Remarks	References
6. <i>Trypanosoma</i> (Pl. II, figs. 1-3)	<i>Bufo melanostictus</i> , <i>B. stomaticus</i> , <i>B. andersoni</i> .	Balitha, Bankura District ; Purulia, West Bengal ; Bondla, Mollem & Volpoi, Goa, India.	Pleomorphic haemoflagellate occurring in 4 Distinct forms ; body leaf-like, flattened and thinned out along the convex edge to form undulating membrane ; cytoplasm densely granular with striated myonemes ; kinetoplast dot-like and situated nearer to the posterior end ; nucleus oval or round and situated on the posterior part of the body ; free flagellum long (11-16 $\mu\text{m}$ ).	It constitutes new host records from Indian sub- continent.	Mayer (1843) Ray (1979a, b, 1980), Ray & Choudhury (1983).
7. <i>Trypanosoma</i> <i>chattoni</i> (Pl. II, figs. 4-6)	<i>Bufo melanostictus</i> , <i>B. stomaticus</i> , <i>B. andersoni</i> .	Volpoi, Mollem, Bondla, Goa, Jalpaiguri, West Bengal, India.	Rounded, spherical or irregular body ; cytoplasm contains innu- merable metachromatic granules and fine vacuoles ; nucleus circular and central ; kinetoplast very small, circular or elliptical and superimposed upon the surface of the nucleus ; flagellum very short either straight or curved and extended to a certain length.	<i>T. chattoni</i> has been recorded from a number of bufonid hosts.	Mathis & Léger (1911), Ray (1979b, 1980), Ray & Choudhury (1983).
8. <i>Microfilaria</i> (larval nematode) (Pl. II, fig. 8)	<i>Bufo melanostictus</i>	Amgram, 24-Parganas, West Bengal, India.	Snake-like, sheathed, long, slender measuring 95-120 $\mu\text{m}$ in length and 5.5-6.5 $\mu\text{m}$ in width.	This sheathed microfilaria is recorded for the first time in toads from India.	Ray (1979b)
9. <i>Microfilaria</i> (larval nematode) (Pl. II, fig. 7)	<i>Bufo melanostictus</i>	Jhargram, Midnapore, West Bengal, India.	Snake-like ; unshathed, measuring 98-125 $\mu\text{m}$ in length and 5-6 $\mu\text{m}$ in width.	—	Nandi and Mandal (1977) Ray (1979b)



- Figs. 1 & 2. Mature gametocytes of *Haemogregarina nucleobisecans* in the peripheral blood of *Bufo melanostictus* X 1665
- Figs. 3 & 4. Mature gametocytes of the same haemogregarine in the blood of *Bufo andersoni* X 1410
- Fig. 5. A multinucleated mature schizont in the liver parenchyma of *B. melanostictus* X 1100
- Fig. 6. A mature schizont with developing merozoites in the liver parenchyma X 1660
- Fig. 7. Free sporozoite of *Lankesterella bufonis* in the blood of *B. melanostictus* X 1130
- Figs. 8 & 9. Intracorpuseular sporozoites of *L. bufonis* X 1180
- Fig. 10. *Pirhemocytos* in *Bufo melanostictus* X 1000
- Fig. 11. The arrow shows the *Pirhemocytos* in the blood of *Bufo hemalayanus* X 1000
- Fig. 12. *Cytamoeba bacterifera* in the blood of *Bufo melanostictus* X 1000



- Fig. 1. *Trypanosoma rotatorium* in the peripheral blood of *Bufo melanostictus* X 1465
- Fig. 2. The same haemoflagellate in *B. stomaticus* X 1225
- Fig. 3. *T. rotatorium* in *B. andersoni* X 1200
- Fig. 4. *Trypanosoma chattoni* in the peripheral blood of *B. melanostictus* X 1225
- Fig. 5. *T. chattoni* in *B. stomaticus* X 1220
- Fig. 6. *T. chattoni* in *B. andersoni* X 1200
- Fig. 7. Photomicrographs of unsheathed microfilaria in the blood of *B. melanostictus* X 545
- Fig. 8. A sheathed microfilaria in the peripheral blood of *B. melanostictus* X 835

*Remarks* : The taxonomic status of *Pirhemocytion* is doubtful even today. Previously it was grouped with the protozoa. Levine (1978, Personal Communication) opined that *Pirhemocytion* are virus inclusion bodies. Stehbens and Johnston (1966) conducted an electron microscopic studies to know the morphology of the parasite. Soltz (1971) included *Pirhemocytion* among a group termed as icosahedral cytoplasmic deoxyriboviruses (ICDV,). In absence of the proper knowledge about the biology of these parasites no name has been proposed.

#### MICROFILARIA (the larval nematode)

*Host* : *Bufo melanostictus*.

#### Sheathed form

(Plate V, Fig. 8)

*Description* : N=20. These microfilariae are sheathed, long slender measuring 95-120  $\mu\text{m}$  in length and 5.5-6.5  $\mu\text{m}$  in width. The sheath is hyaline, measuring 1.5-20  $\mu\text{m}$  in width from the body and often found free at the caudal end. The cephalic space is very small measuring 2.0  $\mu\text{m}$  in length. At the anterior end there are two rows of nuclei, 3 in each row and a gap in between them is well marked. The nuclear column consists of 4-5 rows of closely packed nuclei, more compact at the anterior half. The nuclei stained pink with Leishman's stain. The last nucleus is more or less rounded and located at the tip of the tail. The tail is 10.5-12.2  $\mu\text{m}$  in length, gradually narrowed and ending in a rounded tip. Position of the land marks : nerve ring 25.4-26.0% ; excretory pore 34.8-35.5% ; excretory cell 44.6-45.5% ; inner body 69.1-70.5% ; anus 82.8-84.5%.

*Habitat* : Blood.

*Locality* : Amgram, 24, Parganas district, West Bengal.

*Occurrence* : In one toad out of 343 examined.

*Remark* : Nandi and Mandal (1977) reported one unsheathed microfilaria from *Bufo melanostictus* for the first time in India. In the present investigation the author also observed an unsheathed microfilaria (Fig. 7) which well corroborates with Nandi & Mandal (1977) in all respect. Therefore, the sheathed microfilaria in *B. melanostictus* is recorded for the first time from India. The specific determination of this larval nematode is left without finding adults.

#### SUMMARY

During the survey on amphibian haematozoa, 478 examples of toads comprising 4 species viz., *Bufo melanostictus*, *B. andersoni*, *B. stomaticus*



and *B. himalayanus* were examined of which the first 3 anuran hosts show the infection of *Trypanosoma rotatorium* (Mayer, 1843) and *T. chattoni* Mathis and Léger, 1911 in their blood. The blood and tissue stages of *Haemogregarina nucleobisecans* Shortt, 1917 have also been observed in *B. melanostictus* and *B. andersoni*. A haemococcidia viz., *Lankesterella bufonis* Mansour and Mohammed, 1962 has been recorded in a new host *B. melanostictus* for the first time from Indian subregion. Two microfilariae both sheathed and unsheathed have also been observed in the blood of *B. melanostictus*. Besides, some organisms of uncertain taxonomy viz., *Pirhemocytos*, *Toddia* and *Cytamoeba* have been found to infect *B. melanostictus*, *B. andersoni* and *B. himalayanus*. All those parasites have been enumerated in a table.

#### ACKNOWLEDGEMENTS

The author is very much thankful to Dr. B. K. Tikader, Director, Zoological Survey of India, Calcutta for providing laboratory facilities. Thanks are also due to Dr. Amalesh Choudhury, University of Calcutta, Dr. A. K. Mandal, Superintending Zoologist and Dr. A. K. Das, Zoologist, Zoological Survey of India for their constant guidance and encouragement during the work.

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