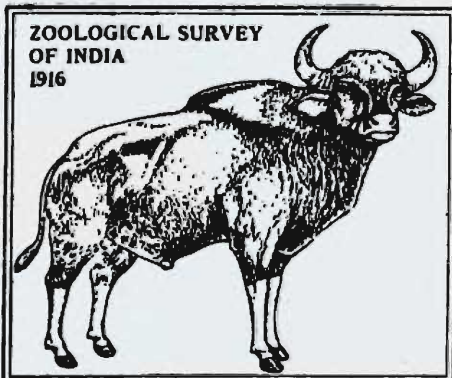


TECHNICAL MONOGRAPH No. 10

HAEMOGREGARINES OF INDIAN ANURANS

by

R. RAY AND A. CHOUDHURY



**ZOOLOGICAL SURVEY
OF INDIA**

Zoological Survey of India

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Edited by the Director, Zoological Survey of India
1984

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Published : September, 1984

PRICE : Inland : Rs. 46.00

Foreign : £ 5.50 \$ 10.00

Printed in India by Saakhhar Mudran, 4, Deshapran Sasmal Road, Calcutta-33
and Published by the Director, Zoological Survey of India, Calcutta.

Zoological Survey of India

TECHNICAL MONOGRAPH

No. 10

1984

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INTRODUCTION

The genus *Haemogregarina* was established in 1885 by Danilewsky for the haemogregarines of the tortoise, which was named *H. stepanowi*. The amphibian *Haemogregarina* was first discovered by Kruse, 1890 in *Rana esculenta* and was named as *Haemogregarina ranarum*. Grassi and Feletti, 1891, recorded it from European frog *R. esculenta* and named it as *Drepanidium magnum*. Labbé (1899) observed the same parasite in the host *Rana ridibunda* and he described it as *Haemogregarina magna*. Since 1890, 13 named and 8 unnamed species of *Haemogregarina* from the family Ranidae and 11 named and 2 unnamed species from the family Bufonidae, are so far reported. Despite this, there exists much controversy regarding their taxonomy (Mohammed and Mansour, 1959) and still little is known of their biology.

In India, Dr. N. Berestneff (1903) was the first man who observed a haemogregarine parasite in the blood of some frogs at Bombay. After that stray reports were made only by a few investigators like Castellani and Willey (1905), Patton (1908), Dobell (1910), De Mello *et. al.* (1917), Shortt (1917), Scott (1926) and Wenyon (1926) in this country from time to time on the blood parasites of Indian amphibians.

Recently Ray (1977, 1979, 1982, 1983 a, b) and Tiwari and Ray (1981) studied the anuran haemogregarines in details from India. Still there seems to have some lacunae in informations. A comprehensive biological monograph on the Haemogregarines of Anuran Amphibians of India may satisfy the demand to some extent. The authors have tried their best to include all the published account of haemogregarines from India. While describing each species all the synonymies have been given along with the recorded references followed by type-host, other host records, distribution, prevalence and morphology of the parasites in detail. The developmental stages of the haemogregarines in the tissue of the vertebrate hosts (Anura) are also included whenever it is available. A key for the identification of species of anuran haemogregarines of India has been given before the description of the species. A remark on the taxonomy of the anuran haemogregarines has also been included at the end of the present treatise.

The authors hope that this monograph on the haemogregarines of Indian Anurans will be great help to those researchers who are working on this particular field of parasitology.

MATERIAL AND METHODS

All the anuran amphibians were collected from different districts of West Bengal, Orissa, Bihar, Assam, Andaman Islands, Tripura, Nova-Goa,

Kerala etc., and examined for haemogregarines. 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 routine examinations.

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 capillary pipette or hypodermic syringe. Impression and spread preparations were made from liver, lungs, kidney, spleen and bone-marrow. Air-dried blood films and organ smears were fixed in 100% methanol and stained with Romanowsky type of stains and examined. For the endogenous stages of the parasite small pieces of tissue of the suspected organs such as lungs, liver, heart, kidney, spleen, brain etc. were dissected out from the sacrificed infected hosts. The cut surfaces were dabbed on filter paper or blotting paper to remove most of the blood and immediately transferred into either alcoholic Bouin's fixative for over night followed by standard histological procedures (Pearse, 1960). Six micron sections were cut for each tissue and the sections were stained with Iron-haematoxylin and counter stained with Eosin.

The morphometric parameters were recorded after Bennett and Campbell (1972) with a little modification. For each species at least 15 parasites and 15 normal erythrocytes or otherwise as mentioned in the description, were measured. Linear dimensions and areas were measured with the help of calibrated oculometer and from the camera-lucida drawings drawn on a graph paper of mm division. By making a camera-lucida drawing of the stage micrometer scale on the same graph paper, the length and area measurements of each square were calculated. Mensural data of the parasites were noted from the drawings with the help of a fine divider which was spreaded at a small unit measurement. The following morphometric parameters were documented (Fig. A).

1. Normal erythrocyte : Length, width and area of erythrocyte ; length, width and area of erythrocytic nucleus, NDR (Nuclear Displacement Ratio).
2. Parasitized erythrocyte : (Fig. A). Same as for normal erythrocyte.
3. Parasite (Fig. A). Length, width and area of parasite ; length width and area of parasite nucleus ; NDR.

The amount of lateral displacement of the erythrocyte nucleus by the parasite was defined by Bennett and Campbell (1972) as the Nuclear Displacement Ratio (NDR). The ratio was obtained by dividing the distance

between the periphery of the cell and the periphery on the nucleus (X) by the distance between the cell and periphery of the nucleus on the other side (Y) and was expressed by the formula $NDR = 2X / X + Y$. In unparasitized erythrocyte, the NDR is unity, the degree of lateral displacement of the nucleus by the parasite is shown by the approximation of the NDR to zero.

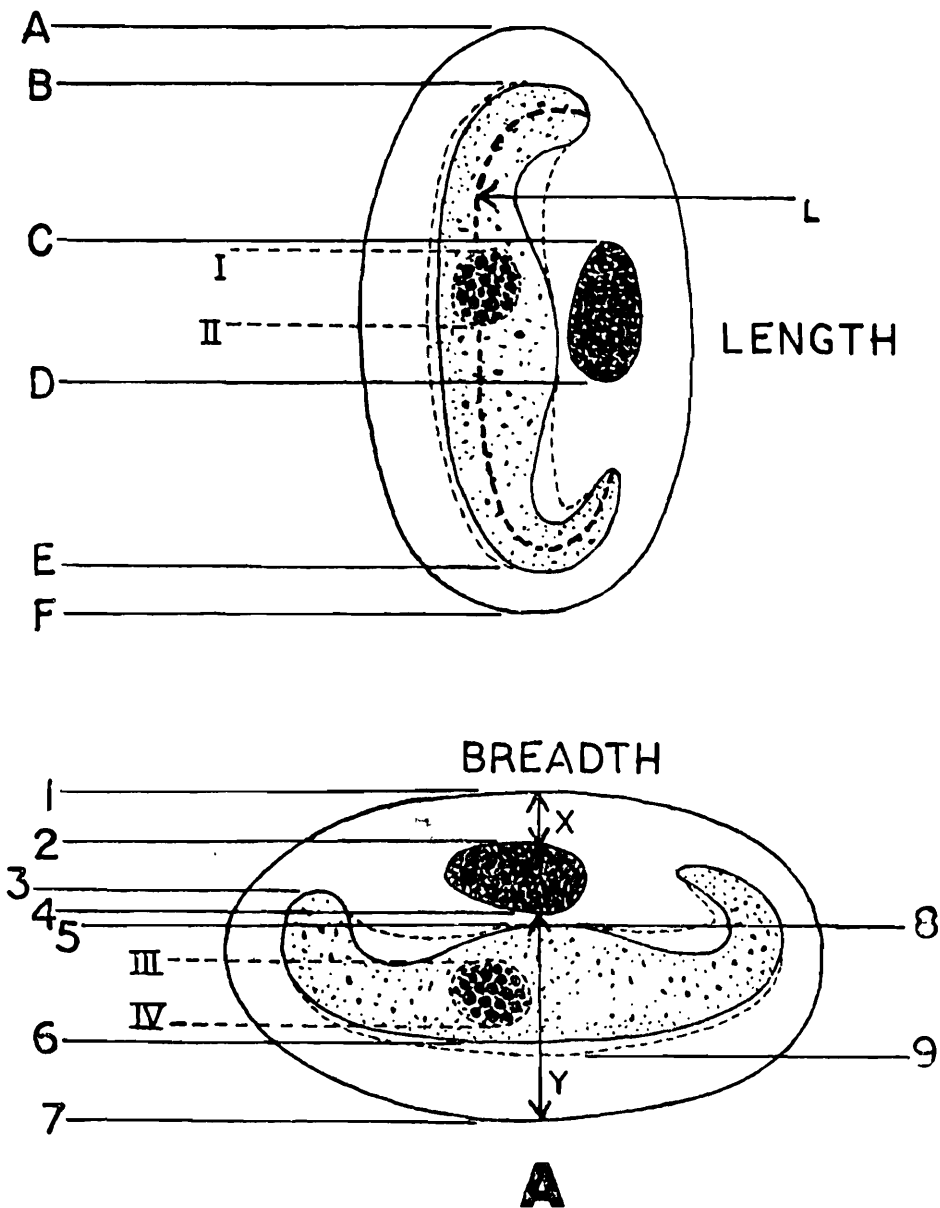


Fig. A. Drawings to illustrate measurements of linear dimensions of *Haemogregarina*.

Length : F—A, the length of host cell ; D—C, the length of host cell nucleus ; E—B, the length of parasite or capsule where the parasite is straight, in bent parasite the length is measured along the broken line 'L' ; I—II, the length of parasite nucleus.

Breadth : 7—1, the breadth of host cell ; 4—2, the breadth of host cell nucleus ; 6—5, the breadth of parasite ; 6—3, the breadth of parasite where it is straight ; 9—8, the breadth of parasite capsule ; III—IV, the breadth of parasite nucleus.

The data were analysed for mean (M), standard deviation (SD, indicated in the text in parentheses), standard error of the mean (SE), coefficient of variation (CV), and co-relations between various parameters were made.

The photomicrographs were taken with the help of "Ergavel C. Z. microscope" using PM6 Olympus attachment camera. The type slides will be deposited to the National Zoological Collection of the Zoological Survey of India, Calcutta.

Key to the Identification of Anuran Haemogregarines in India

- I. Capsule and polar cap present
- a) size of gametocyte $14.40 \times 2.95 \mu\text{m}$ and nucleus $3.25 \times 1.95 \mu\text{m}$; host cell hypertrophy in area 11.3%... *H. nucleobisecans*
- b) size of gametocyte $16.02 \times 3.65 \mu\text{m}$ and nucleus $3.90 \times 2.06 \mu\text{m}$; host cell hypertrophy in area 7.5%... *H. kaloulae*
- c) size of gametocyte $15.30 \times 3.90 \mu\text{m}$, and nucleus $3.40 \times 2.40 \mu\text{m}$; host cell hypertrophy in area 13.7% ... *H. systoma*
- II. Capsule and polar cap absent
- a) size of gametocyte $12.40 \times 4.06 \mu\text{m}$ and nucleus $4.40 \times 3.30 \mu\text{m}$; host cell hypertrophy in area 1.3% .. *H. magna*
- b) size of gametocyte $11.10 \times 3.10 \mu\text{m}$ and nucleus $3.40 \times 2.20 \mu\text{m}$; host cell hypertrophy in area nil ... *H. perinucleophilum*
- c) size of gametocyte $14.55 \times 3.45 \mu\text{m}$ and nucleus $2.05 \times 1.70 \mu\text{m}$; host cell hypertrophy in area 33.1% .. *H. andamanensis*
- d) size of gametocyte $15.41 \times 3.0 \mu\text{m}$ and nucleus $2.33 \times 1.75 \mu\text{m}$; host cell hypertrophy in area 13.7% ... *H. pulchra*
- III. Capsule present but polar cap absent
- a) size of gametocyte $16.50 \times 2.57 \mu\text{m}$ and nucleus $2.85 \times 2.19 \mu\text{m}$; host cell hypertrophy in area 16.6%... *H. berestneffi*
- b) size of gametocyte $9.70 \times 4.93 \mu\text{m}$ and nucleus $3.42 \times 2.50 \mu\text{m}$; host cell hypertrophy in area 44.2% ... *H. pattoni*
- IV. Capsule absent but polar cap present
- a) size of gametocyte $16.50 \times 3.30 \mu\text{m}$ and nucleus $2.40 \times 2.10 \mu\text{m}$; host cell hypertrophy in area 3.13% .. *H. maculatus*

GENERAL ORGANISATION

Genus *Haemogregarina* Danilewsky

Morphology: Parasites of the erythrocytes in the circulatory system of vertebrate; schizogony takes place in the red blood-corpuscles or other cells of the body of vertebrates; merozoites escape from the original host-cell and infect other corpuscles. After several generations merozoites enter red-blood corpuscles and become micro and macro-gametocytes. The gametocytes of both the sexes can be differentiated by the structure of their nucleus and tinctorial property of the cytoplasm. The gametocytes are elongated with the anterior end more or less rounded and the posterior end narrower or more pointed. Sometimes the posterior end is folded on itself in such a way so as to form a loop. The various postures taken by the

parasite probably depend on its length in relation to the size of the host cell. The gametocytes may be either enclosed in a colourless capsule or the capsule may be absent in some cases.

Life-cycle : The gametocytes are taken up with the blood meal by an invertebrate vector (Leech). The gametocytes escape from their host-cells, become more or less spherical, associate in pairs, and a cyst-wall is formed, enclosing both the macro and microgametocyte. The microgametocyte produces 2—4 microgametes, and the macrogametocyte forms a single macrogamete. One of the microgamete fertilizes the adjacent macrogamete, and a small oocyst is formed. The oocyst directly produces eight sporozoites without the formation of sporocyst. The whole development takes place in side the gut of the leech-vector. The life-cycle was described by Reichenow (1910).

SYSTEMATIC DESCRIPTION OF THE SPECIES OF HAEMOGREGARINA
Order EUCCOCIDA Léger and Dabsocq
Suborder ADELEINA Léger
Family HAEMOGREGARINIDAE Neveu-Lemaire

Diagnosis ; Oocyst without sporocyst. The schizogony or asexual multiplication occurs in the red blood-corpuscles or other cells of the body of vertebrates. After several generations merozoites enter red blood corpuscles and become micro or macrogametocytes ; transmitted by invertebrate vector (Leech) in the gut of which association of micro-and macrogametocytes takes place. After fertilization a small oocyst is formed.

Genus *Haemogregarina* Danilewsky

1885. *Haemogregarina* Danilewsky, *Arch. Mikr. Anat.*, 24 : 588.
1894. *Danilewskya* Labbé, *Arch. Zool. exp. gén.*, 3 : 124.
1899. *Haemogregarina* Labbé, *Sporozoa* (In *Das Tierreich*. Berlin) p. 76 ; Reichenow, 1910, *Arch. Protistenk* ; 20 : 251 ; Minchin, 1912, *An introduction to Protozoa*, London p. 372, Wenyon, 1926, *Protozoology*, Bailliére, Tindal and Cox London, p. 1081 ; Calkins, 1933, *Biology of the Protozoa*, 2nd Edn., Philadelphia and New York pp. 545, 567 ; Kudo, 1966. *Protozoology*, 5th Edn., Thomas, Springfield, Illinois, 1174 pp.

Diagnosis : Schizogony in the red blood corpuscles or in the internal organs of a vertebrate ; gametogony and fertilization in the body of blood-sucking invertebrate (Leech) ; oocyst small, directly producing eight sporozoites, without forming sporocyst.

Type-species : *Haemogregarina stepanowi* Danilewsky.

(This species was described from an European tortoise *Emys orbicularis* (Linn.)

ANURAN HAEMOGREGARINES

1. *Haemogregarina berestneffi* Castellani and Willey (Plate I Fig. 1—13 and Figs. 1a—n)

1903. *Haemogregarina encapsulae*, Berestneff, *Arch. Protistenk.*, 2 : 343.
 1905. *Haemogregarina berestneffi* Castellani and Willey, *Q. J. Microsc. Sci.* 49 : 383 ; Dobell, 1910, *Spolia Zeylan.*, 7 : 65 ; de Mello *et al.*, 1917, *Ann Sci. (Faculd. Med.) Porto.*, 3 : 5 ; Wenyon, 1926, *Protozoology* Bailliére, Tindall and Cox, London, p. 1398 ; Scott, 1926, *Proc. zool. Soc. Lond.*, 1 : 238 ; Bhatia, 1938, *The Fauna of British India, Protozoa : Sporozoa*, Taylor and Francis, London, p. 119 ; Ray, Ph. D. Thesis, Calcutta University, 1983b, *Rec. zool. Surv. India*, 81 : 55.
 1908. *Haemogregarina* sp., Patton, *Parasitology*, 1 : 318.

Type-host : *Rana tigrina* Daudin.

Type-locality : Ceylon,

Other hosts : *Rana limnocharis* Wiegmann.

Rana hexadactyla Lesson (Both from India).

Present new host record : *Rana cyanophlyctis* Schneider.

Locality : Madras, Bombay, Nova Goa ; Balitha, Bankura District, West Bengal, India.

Prevalence : 37 out of the 307 examples of *Rana limnocharis* and 5 out of 450 *R. cyanophlyctis* examined from different parts of India, were found positive. Almost 35% of the total R.B.C. counted were found to be infected with this parasite.

Description : The following description is based on the parasite obtained from *Rana limnocharis*.

Gametocytes :

- 1) Elongate mature form (Fig. 1a, b ; Plate I, Fig. 4, Table 1) :

N=15. Most parasites have typical elongate straight "haemogregarine" shape, with the anterior end more or less rounded, and the posterior end narrower and more pointed. Sometimes the posterior end is folded on itself in such a way so as to form a loop. The various postures taken by the parasite probably depend on its length in relation to the size of the host cell. The elongate form is 16.5 μm (0.76) long

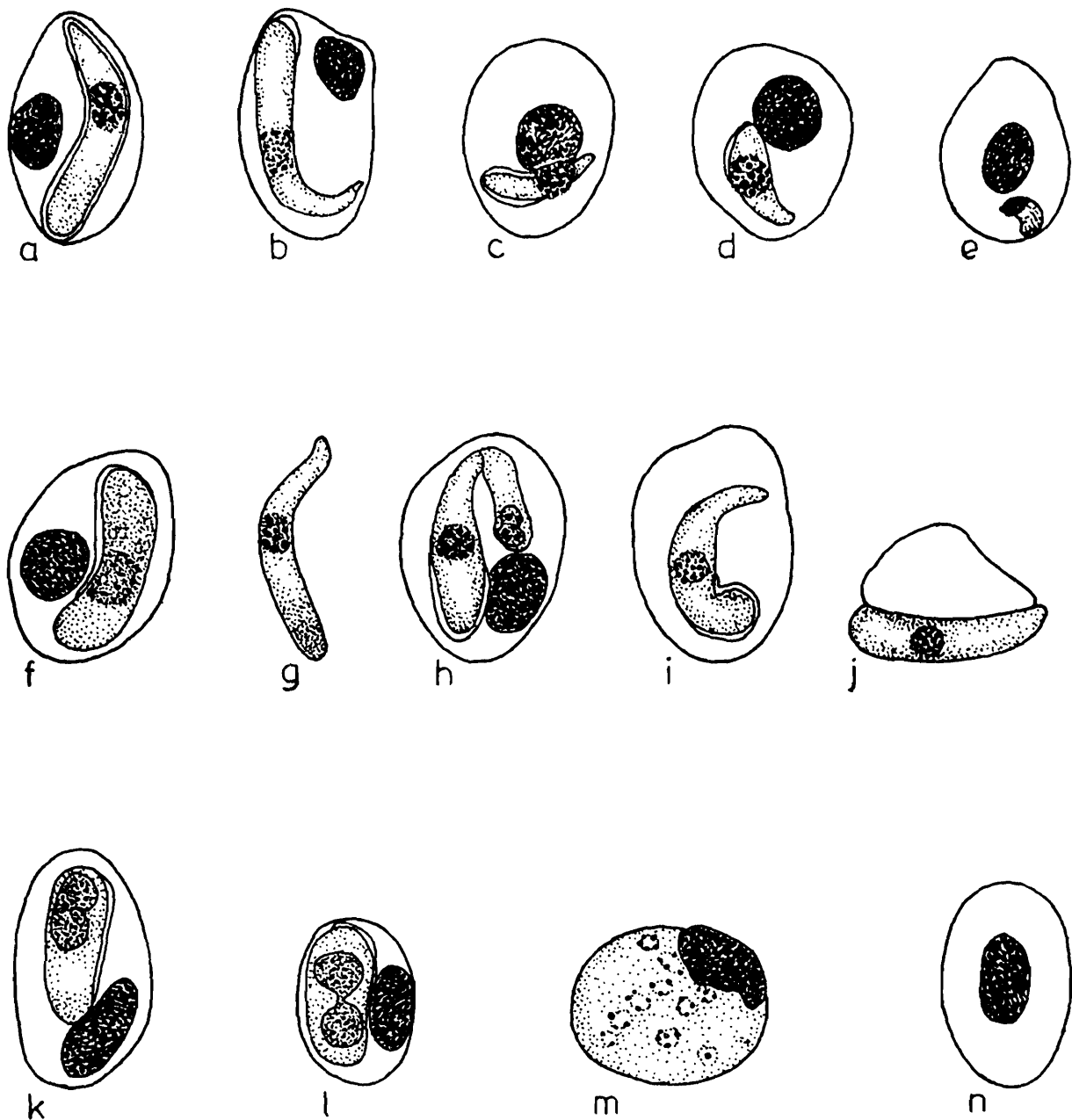


FIG.1 HAEMOGREGARINA BERESTNEFFI.

10µm

Fig. 1. Camera lucida drawings of the erythrocytic stages of *Haemogregarina berestneffi* from *Rana limnocharis*.

a—j, Gametocyte stages. a, the macrogametocyte ; b, the microgametocyte ; c & d, the young-broad forms ; e, the young thin forms ; f, the growing form where the nucleus become fragmented and cytoplasm become vacuolated ; g, the extra-corporal free form ; h, the double infection with a mature form and an young form ; i & j, the enucleated R.B.C. containing mature gametocyte ; k-m, Erythrocytic schizonts. k, the young schizonts ; l, the schizont with dividing nucleus ; m, the mature schizont with 10 nuclei ; n, the uninfected erythrocyte.

and $2.57 \mu\text{m}$ (0.32) wide with an average of $29.56 \mu\text{m}^2$ (3.18) in area; occupying about 29% of host cell-parasite complex. Cytoplasm homogeneous, scantily granular at the anterior end; stained pinkish blue with Giemsa and Leishman stain.

The nucleus is round, oval or irregular, usually central or a little below towards the posterior end. It is $2.85 \mu\text{m}$ (0.39) by $2.19 \mu\text{m}$ (0.22) with an average area about $5.13 \mu\text{m}^2$ (0.76); stained purple with Leishman and Giemsa and contained compact network of granules. The parasites are enclosed within a thin distinct pink staining, closely fitting capsule measuring $18.3 \mu\text{m}$ (1.60) by $3.14 \mu\text{m}$ (0.27).

On the basis of the tinctorial property and structure of the nucleus gametocytes may be differentiated into macrogametocytes with deep pink staining granular cytoplasm and a compact rounded nucleus where as microgametocyte (Fig. 1b) with comparatively lighter, clear cytoplasm and band-shaped nucleus. Parasites enucleating infected host cells (Figs. 1i, j) are rarely be seen. Double infection (Plate 1 Fig. 7) of the R.B.Cs was observed in acute cases. The two co-habitants crowd the host cell and arrange themselves in one way or another along the length of the much distended corpuscle (Fig. 1h).

2) Young and growing forms (Figs. 1c—f, Plate 1 Figs. 2, 6.) :

Young broad forms : This form is broad at one end and tapering at the other. The nucleus is flower-shaped, large, rounded and almost covering the total width of the body. They are lying either on the concave border of the host cell nucleus or sometimes in association with larger elongated form. This form measures $7.34 \mu\text{m}$ (1.46) by $2.11 \mu\text{m}$ (0.16) and $12.05 \mu\text{m}^2$ (3.3) in area. During growth, it becomes slightly curved, the cytoplasm becomes vacuolated, and the nucleus differentiates into transverse strands or dots. The nucleus, which grows slightly in length but more in width, fills the breadth of the parasite, and nuclear material becomes more dense at the two sides (Fig. 1f). As growth continues, fairly large forms, approximately $14.24 \mu\text{m}$ (0.8) by $3.92 \mu\text{m}$ (0.32) and $47.6 \mu\text{m}^2$ (1.85) in area are produced.

Young thin forms : The young thin form is somewhat ‘,’ shaped with two ends more or less rounded; situated somewhat away from the host cell nucleus. It measures $3 \mu\text{m}$ by $2 \mu\text{m}$ and an area about $2.5 \mu\text{m}^2$. The nucleus is rounded and very small measuring $1 \mu\text{m}$ in diameter (Fig. 1e).

Effect of the parasite on the host cell : Erythrocyte nucleus is markedly placed at one side at the periphery ; the posterior end of the parasite may be bent away from or more frequently towards the host cell nucleus. Less frequently the nucleus may be displaced to the apical end of the cell or sometimes it being totally rejected. $NDR=0.23$ (0.08). Host cell hypertrophied in length (8.8%), width (15.3%) and in area (16.6%). Host cell nucleus hypertrophied in area (7.1%).

Movement in fresh preparation : When a fresh sample of blood mixed with citrated saline solution, is examined, some parasites were seen to excapsulate, escape from their host-cells and move slowly in the fluid with its broader anterior end gliding forward. Some were also seen to invade new host cells. Special organelles or undulations causing movement were not observed.

Extracorpuseular free form (Fig. 1g, Plate I Figs. 8 & 9) : Elongated gregariniform with anterior end broader and attenuated posterior end. They measure $17.6 \mu\text{m}$ (0.74) by $2.3 \mu\text{m}$ (0.22) with an area about $27.7 \mu\text{m}^2$ (1.28). The nucleus is a round mass situated centrally or a little below towards the posterior tapering end ; measuring $2.66 \mu\text{m}$ (0.45) by $2.25 \mu\text{m}$ (1.0) with an average area of $4.77 \mu\text{m}^2$ (1.34) ; stained deep pink with Leishman and Wright. Sometimes they are found either invading the host cell or coming out of it. In citrated saline preparation they exhibit a beautiful gliding forward movement under phasecontrast microscope.

Uninfected erythrocytes : $N=15$. Cell $12.9 \mu\text{m}$ (0.37) by $9.1 \mu\text{m}$ (0.53) and $86.97 \mu\text{m}^2$ (9.41) in area. Cell nucleus 5.3 (0.6) by $3.14 \mu\text{m}$ and $31.5 \mu\text{m}^2$ (4.06) in area. $NDR=0.95$ (0.05).

Schizogony : Developmental stages of *Haemogregarina berestneffi* have not been reported earlier. After repeated and extensive search during the present study, desired schizogonic stages were encountered both in the erythrocytes and tissue section of lungs of the anuran hosts.

Erythrocytic schizogony (Figs. 1k—m, Plate 1, Fig. 4) : In the peripheral circulation a large number of erythrocytic schizonts have been detected. During the initial stage of division the parasite nucleus divides and produces two nuclei at the two polar regions of the parasite. As further division proceeds the nuclei arrange haphazardly throughout the vacuolated light stained cytoplasm. At this stage the nuclei stain purple red and is made up of 3—4 chromatin granules. Bi- and quadri nucleated schizonts are of common occurrence. The maximum number of nuclei detected in an

erythrocytic schizont are upto 10 in number where the host cell becomes very much hypertrophied both in length and width and sometimes it may burst thereby liberating the merozoites measuring $5\ \mu\text{m}$ by $1\ \mu\text{m}$. The merozoites are slender with one end little broader than the other; cytoplasm coarsely granular; stained purple; nucleus is just like an aggregation of 2 or 3 chromatin dots.

Tissue Schizogony (Plate 1, Figs. 10—13): One type of schizont was recognized in the histological section of lungs only. Whereas the liver, kidney and spleen remain uninfected. A large number of schizonts have been detected in the endothelial cells of the lung capillaries. The earliest developmental stage is an oblong or spherical uninucleated body occupying a definite parasitophorous vacuole within the lung endothelial cell; measuring $6.8\ \mu\text{m}$ in diameter. The nucleus stained deep black and cytoplasm stains light blue containing fine granules with Iron-haematoxylin stain. The large type of mature schizont measures $28.9\ \mu\text{m}$ by $17\ \mu\text{m}$ with closely packed nuclei (maximum number averages 35—70) around a broad residual body about $9.5\ \mu\text{m}$ in diameter (Plate 1 Fig. 12). Intermediate stages between this early form and the mature schizont generally follow the same pattern but the nuclei are arranged in a ring around a large residual mass (Plate 1 Fig. 10). Later on, these larger schizonts give rise to the merozoites which are closely packed in a ring around a broad residual body measuring about $10\ \mu\text{m}$ in diameter (Plate 1 Fig. 11). The merozoites are curved, relatively slender and averages $5.2\ \mu\text{m}$ by $1.7\ \mu\text{m}$ (Plate 1 Fig. 13). One end is more broader than the other which is somewhat narrower. The compact round nucleus which stained deep black situated either centrally or towards the broader end.

Remarks: Berestneff (1903) recorded the parasite from the blood of *Rana tigrina* but he did not give any specific name. He stated that “the parasite is enclosed within a strongly refringent colourless capsule both ends of which are rounded and curved, the whole embracing the displaced nucleus of the corpuscle after the manner of a Halteridium” He also stated that when the infected R.B.C. broke up, the parasites were released into the plasma, become straight and then began to move by rounded anterior end projecting forward. Castellani and Willey (1905) opined that Berestneff’s parasite differs from the parasite described by them in respect of two points—by the relation of the merozoite to its capsule and by the position of the nucleus. As there are some close similarities so they described the Berestneff’s species as *Haemogregarina berestneffi* after the name of Dr. N. Berestneff who first recorded the parasite from the blood of *Rana tigrina*. The present parasite is well corroborated with the description of Berestneff

(1903) and Castellani and Willey (1905) but differs in not having the club shaped empty end of the capsule. Here the capsule is pink coloured, closely fitted, leaving no space and the position of the nucleus is either central or little below towards the attenuated end. The parasites described in the present paper is little smaller in size. Sometimes the parasite made a loop on itself causing great hypertrophy to the host cell both by length and width. Donovan (cited from Wenyon, 1926), Dobell (1910), Patton (1908) and de Mello *et. al.* (1917) also recorded the same species from *Rana tigrina* of Ceylon and *R. tigrina* and *R. hexadactyla* from Madras and *Rana tigrina* from Nova Goa respectively but their descriptions were inadequate.

In the present communication *H. berestneffi* has been discovered from *Rana cyanophlyctis* for the first time from Indian sub-region where it measures 11.5 μm (0.79) by 2.06 μm (0.79) with an area of 36.15 μm^2 (4.14), which is rather short in measurement. The capsule is also pink-coloured and well marked, measuring 12.5 μm (0.61) by 3.8 μm (1.31) with an area about 36.95 μm^2 (5.04). The morphometric parameters of the *Haemogregarina* from two host species viz., *R. limnocharis* and *R. cyanophlyctis* are presented in Table 1.

The erythrocytic schizogony and the tissue schizogony of this haemogregarine are of special interest and described for the first time from India.

The asexual multiplication is observed in the peripheral blood and in the blood obtained from lung smear. The binucleated schizont with two polarised nuclei and quadrinucleated schizonts are recorded in innumerable number. Each schizont becomes spherical almost filling the total area of the host cell and thereby pushing the host cell nucleus at one corner.

Various schizogonic stages of *H. berestneffi* were detected in the endothelial cells of lung capillaries which appeared to be the only site of multiplication. None were seen in other organs (liver, heart, kidney, spleen, pancreas etc.). Mature schizonts produce oblong banana-shaped merozoites which, after some growth in the erythrocytes, appear in the peripheral blood as young broad forms.

2. *Haemogregarina magna* (Grassi & Feletti)

(Figs. 2a—j, Plate II Figs. 1—13)

1890. *Haemogregarina ranarum* (in part), Kruse, *Virchow. Arch. Path. Anat. Physiol.*, 10 : 541 ; Celli and Sanfelice, 1891, *Ann. Agric. Roma.*, 183 : 504.
 1891. *Drepanidium magnum* Grassi & Feletti, *Cent. f. Bakl. u. Parasit.*, 10 : 449 ; 1892, *Atti Accad. Gioenia Sci. Nat. Catania* V. i, Mem. 5. p. 81.

1894. *Drepanidium krusei* Labbé, *Arch. Zool exp. gen.*, 2 : 54 ; Berestneff, 1903, *Arch. Protistenk.*, 2 : 347.
1899. *Haemogregarina magna* Labbé. *Sporozoa*. IN : *Das Tierreich*. Schulze, F. F. and O. Butschli, eds. Vol. 5 : p. 76 ; Patton, 1908, *Parasitology*, 1 : 318 ; Wenyon, 1926, *Protozoology*, Bailliére, Tindal and Cox, London, p. 1398 ; Bhatia, 1938, *Fauna of British India, Protozoa, Sporozoa*, Taylor and Francis, London, p. 124 ; Ray, 1979, Ph. D., Thesis, Cal. Univ., 1983b *Rec. zool. Surv. India*, 81 : 55.

Type-host : *Rana esculenta* Linn.

Type-locality : Europe

Other hosts : *Rana tigrina*, *R. limnocharis* (both from India), *R. pipiens*, *R. p. berlandieri*, *R. ridibunda*.

Locality : Madras ; Bombay ; Bishnupur, Bankura District, West Bengal, India.

Prevalence : 29 out of 164 examples of *Rana tigrina* examined from different parts of India were found infected. A minimum of 5% to a maximum of 33.5% of the total R.B.C. counted were found to be positive for *Haemogregarina magna*.

Description : The present description is based on the parasite obtained from *Rana tigrina*.

Gametocytes : Elongate mature form (Figs. 2a, b, Plate II Figs. 1—4) : N=15. Gamonts are elongated with broader rounded anterior end and narrower posteriorly. Sometimes the posterior end is folded in accordance with the host cell and occupies a maximum area of the erythrocytes. The parasites are placed either with the concave border of the host cell nucleus or sometimes remain at one pole of the host cell. The mature elongated form is 12.4 μm (1.97) by 4.06 μm (0.23) with an average area of 44.5 μm^2 (7.09) occupying about 42.7% of the total host cell-parasite complex. Cytoplasm homogeneous, hyaline little granular ; stained pinkish blue with Giemsa and blue in Wright. In Iron-alum haematoxylin stain some dark blue granules appear to be uniformly distributed throughout the cytoplasm. The nucleus is broad to oval, sometimes rounded or band-shaped. The position of the nucleus is either central or always towards the broader anterior end. It measures 4.4 μm (0.67) by 3.3 μm (0.28) with an area about 12.1 μm^2 (1.93) ; stained purple or reddish with Romanowsky type of stains ; contains compact chromatin granules.

The parasite was never found to be enclosed in a capsule.

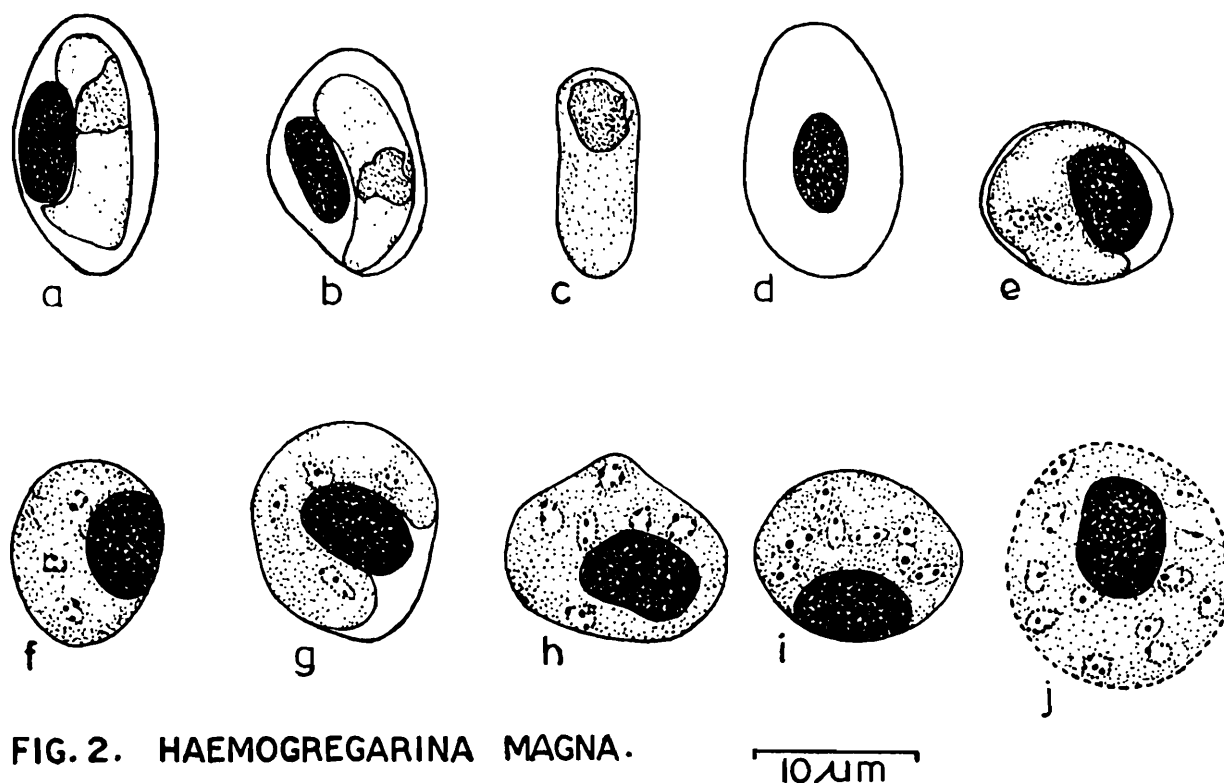


FIG. 2. HAEMOGREGARINA MAGNA.

10 μm

Fig. 2. Camera lucida drawings of the erythrocytic stages of *Haemogregarina magna* from *Rana tigrina*.

a—c, Gametocyte stages. a & b, the mature macrogametocytes ; c, the extracorporeal free gametocyte ; d, the uninfected erythrocyte ; e—j, Erythrocytic schizonts. e, the binucleated schizont ; f, the trinucleated schizont ; g, the quadrinucleated schizont ; h, the hexanucleated schizont ; i, the mature schizont with eight developing merozoites ; j, the mature schizont with 12 nuclei.

Mature gametocytes may be differentiated into male or microgametocytes and female or macrogametocytes on the basis of the tinctorial property and the structure of the nucleus. Both male and female gametocytes are approximately similar in measurement. Microgametocytes (Plate II Fig. 4) are a little slender having a prominent curved tail ; cytoplasm is very much clear with vesicular nucleus where the chromatin materials are very loosely arranged.

Extracorporeal free form (Fig. 2c, Plate II Fig. 6) : N=15. In some cases free forms are seen in the blood plasma. They are elongated with both the ends rounded, anterior end a little broader than the posterior, measuring $11.4 \mu\text{m}$ (1.68) by $4.16 \mu\text{m}$ (0.23) with an average area of $47.7 \mu\text{m}^2$ (6.81). The nucleus is a compact, rounded or oval mass always situated towards the anterior side ; measuring $4.36 \mu\text{m}$ (0.69) by $3.25 \mu\text{m}$ (0.29) and

an area of $12.21 \mu\text{m}^2$ (2.05). The cytoplasm of the free parasites stained light blue with some pink staining granules at one end. The nucleus stained red or purple with Wright and Leishman.

Effect of the parasites on the host cell: Infected erythrocytes were frequently hypertrophied. The host cell nucleus was distorted and elongated, usually displaced to the periphery. $\text{NDR}=0.22$ (0.15). Host cell hypertrophied in length (3.3%), in width (21.1%) and in area (1.3%). Host cell nucleus hypertrophied in area (50%).

Movement in fresh preparations: In citrated saline solution the parasites exhibit a beautiful slow gliding movement with its broader anterior end forward. Sometimes it has been seen that the elongated forms are wriggling out of its host cell into the plasma.

Uninfected erythrocytes (Fig. 2d): $N=15$. Cell $15.6 \mu\text{m}$ (1.26) by $8.5 \mu\text{m}$ (0.52) and $94.4 \mu\text{m}^2$ (12.95) in area. Cell nucleus $5.4 \mu\text{m}$ (0.48) by $2.7 \mu\text{m}$ (0.40) and $12.2 \mu\text{m}^2$ (1.92) in area. $\text{NDR}=0.92$ (0.06)

Schizogony: Series of examination and repeated search revealed two types of schizogony both in the R.B.C. and in the endothelial cells of the lung tissue.

Erythrocytic schizogony (Figs. 2e—j; Plate II Figs. 7—9.): The most interesting part of the study is the revelation of series of erythrocytic schizogony which have been observed both in the peripheral blood and blood obtained from the lung's smears. The erythrocytic schizont measures $12.5 \mu\text{m}$ (1.21) by $10.70 \mu\text{m}$ (1.43) with an area about $103.9 \mu\text{m}^2$ (7.03). Binucleate, trinucleate and tetranucleate schizonts were found abundantly. The number of nuclei in a schizont was recorded up to 12.

A count of 100 schizonts from 5 slides shows the following percentage of merozoites.

2 merozoites	—	49%
3	„	— 14%
4	„	— 6%
6	„	— 1%
8	„	— 6%
10	„	— 16%
12	„	— 8%

Schizonts, mainly the mature one, were found like halteridium as it progressively encircled the host cell nucleus leaving no erythrocytic cytoplasm. The host cell and the nucleus were very much hypertrophied even

during the early stages of development. During this time the cytoplasm of the parasite seemed less compact and thin with fine granules, staining blue and uniformly distributed. Schizont nucleus was the aggregation of 3—4 chromatin materials haphazardly arranged ; stained reddish in Wright and Giemsa. Sometimes the host cell became so much hypertrophied that led it to burst. In the primary stage or in immature schizont a short constriction appeared in the nucleus which gradually differentiated into a binucleated schizont.

Merozoites : Some of the mature schizonts possessed fully developed spindle-shaped merozoites measuring $1.9 \mu\text{m}$ (0.16) by $1.05 \mu\text{m}$ (0.15) with an area about $1.3 \mu\text{m}^2$ (0.21). Each merozoite with narrow ends stained reddish purple ; nucleus either central or subcentral in position and displayed an extranuclear deposition at one end.

Tissue schizogony (Figs. 10—13, Plate II) : Schizonts or meronts were found in the endothelial cells or free in the capillaries of the lung tissue. Liver, spleen, kidney etc. remained negative for the infection. The schizonts displayed spherical to subspherical in configurations. The earliest development stage was an oblong or spherical uninucleated body, occupying a parasitophorous vacuole. These bodies ranged in size $5—10 \mu\text{m}$ by $4.0—9.5 \mu\text{m}$. The nucleus is spherical, either central or at the periphery, measuring $2—3.5 \mu\text{m}$ in diameter, stained deep black in Iron-haematoxylin ; cytoplasm is granular.

The mature schizont is also spherical to sub-spherical measuring $19.5—42 \mu\text{m}$ by $19.0—43 \mu\text{m}$ with an average of $13.57 \mu\text{m}$ and containing 20—30 nuclei or sometimes more. Here the parasitophorous vacuole is not so large like the younger stage. This is probably due to the greater accommodation in the endothelial cells. A residuum of about $1.2 \mu\text{m}$ diameter is occasionally observed surrounding which the rosette of nuclei is formed.

Merozoites : The mature schizont contains merozoites which are not arranged in any particular order. They were elongated, lanceolate, straight or curved bodies measuring $5 \mu\text{m}$ by $1.3 \mu\text{m}$; cytoplasm light, nongranular ; nucleus is almost subcentral, displaced somewhat to the broader end of the parasite. The nucleus is less compact and measures $2.5 \times 1.2 \mu\text{m}$. Some extra nuclear eosin positive bodies are always found at the pointed end of the merozoite just below the nucleus.

Remarks : This parasite was first discovered by Kruse 1890 and named *Haemogregarina ranarum*. Grassi and Feletti 1891 recorded it from European frog *Rana esculenta* and they named it as *Drepanidium magnum*.

Labbé (1899) observed the same parasite in the host *Rana ridibunda* and he described it as *Haemogregarina magna*. In the year 1903, Berestneff recorded the same parasite from Indian frog, however, he unknowingly named the parasite as *Danilewskya krusei*. Later on Patton (1908) recorded *Haemogregarina magna* in *Rana tigrina* from Madras. He stated the parasite as uncap-sulated which was the only distinguishable feature from *H. berestneffi* Castellani & Willey. Wenyon (1926) also observed the parasite from the same host in India.

Recently Levine and Nye (1977) observed this parasite in 44 *R. pipiens* (including 12 *R. p. berlandieri*) in Illinois, Louisiana, New York, Tennessee and Wisconsin. They described both the erythrocytic gamonts and tissue stages in the liver.

Although a number of workers recorded this parasite from different hosts and from different parts of the world, yet they failed to substantiate the species description with quantitative measurements except Levine and Nye (1977) who published for the first time with an elaborate account on the schizogony in the liver tissue. Morphometric measurements (Table—2) recorded by the present authors for the parasite obtained from *Rana tigrina* from West Bengal, India, are included herein and reported for the first time the erythrocytic schizogony of the parasite, which is the most important distinguishing characteristic feature in the taxonomy of *Haemogregarina*. The parasite under discussion agrees well with the description of Levine and Nye (1977) but seems to be smaller in morpho-metric measurements (Table—3). Levine and Nye (1977) described the asexual multiplication of the parasite from the parenchyma cells of the liver. The present authors recorded the schizogony both in the erythrocyte and endothelial cells of the lung capillaries where a large number of immature and mature schizonts were noted. The eosinophilic globular body of the merozoites observed by Levine and Nye was also observed by the present authors who found this globular body in almost all the merozoites within the lung tissue.

3. *Haemogregarina nucleobisecans* Shortt

(Figs. 3a—j ; Plate III Figs. 1—10).

1917. *Haemogregarina nucleobisecans* Shortt, *Indian J. Med. Res.* 4 : 402 ; Bhatia, 1938, *The Fauna of British India, Protozoa : Sporozoa*, Taylor and Francis, London, p. 131 ; Ray, 1979, Ph. D. Thesis Cal. Univ. Ray, 1982, *Proc. IV. Natl. Cong. Parasitol.* Aligarh, p. 10.
1926. *Haemogregarina bisecans* Wenyon, *Protozoology*, Bailliére, Tindal and Cox, London, p. 1397.
1926. *Haemogregarina* sp. Wenyon, *Protozoology*, Bailliére, Tindall and Cox, London, p. 1397 ; Scott, 1926, *Proc. zool. Soc. Lond.* 1 : 231.

Type-host : *Bufo melanostictus* Schneider.

Type-locality : Cawnpore, Delhi and Ambala (Punjab), India.

Present new host record : *Bufo andersoni* Boulenger.

Locality : Purulia district, West Bengal, India.

Prevalence : 47 out of 343 examples of *Bufo melanostictus* and 7 out of 25 *B. andersoni* examined were found positive. A minimum of 5% to a maximum of 15% of the total R.B.C. counting were found to be infected with *H. nucleobisecans*.

Description : The present description is based on the parasite obtained from the type host *Bufo melanostictus*.

Gametocytes : 1) Elongate mature form (Figs. 3a—d, f—h, Plate III Figs. 2 & 3) : N=15. These are sausage shaped endoparasites of erythrocytes, slightly curved and lying with its concave border applied to the convexity of the host cell nucleus or sometimes with its concave border to the convexity of the nucleus. In some cases they may be found at one end of the erythrocyte. The anterior end is regular, rounded and broad, and the opposite end is irregular, narrow and more pointed. The posterior end is usually slightly bent (Fig. 3a) or hook-like (Fig. 3b), or attenuated and folded or more rarely sharply bent on itself. In some cases both the extremities may be recurved and afflicted or the parasite may show a constriction about its centre. The nucleus may lie in the bulged portion on either side of the constriction. These elongate forms measure $14.4 \mu\text{m}$ (1.17) by $2.95 \mu\text{m}$ (0.26) with an average area of $28.95 \mu\text{m}^2$ (3.77) occupying about 25.97% of the total host cell parasite complex. The largest one measures $18.0 \mu\text{m}$ by $3.5 \mu\text{m}$. Cytoplasm stains an azure blue colour or faint pink with Giemsa stain. Progressive staining reveals some metachromatic granules evenly distributed throughout the cytoplasm. The nucleus, approximately central in position almost fills up the entire width of the parasite. It may be rounded (Fig. 3f), oval (Fig. 3a), band-shaped or triangular (Fig. 3h) or irregular in out line, measuring $3.25 \mu\text{m}$ (0.81) by $1.95 \mu\text{m}$ (0.41) with a mean area of $5.18 \mu\text{m}^2$ (1.46); stained dark pink with Giemsa. The nucleus contains a close network of granules and no karyosome could be detected.

The parasites are enveloped in a pink coloured thick capsule, the two poles of which sometimes exhibit considerable vacant space giving the appearance of a cap. In the folded or bent parasite the capsule is very much clear. It measures $15.17 \mu\text{m}$ (1.82) by $3.7 \mu\text{m}$ (0.40) with an area of $31.84 \mu\text{m}^2$ (4.28).

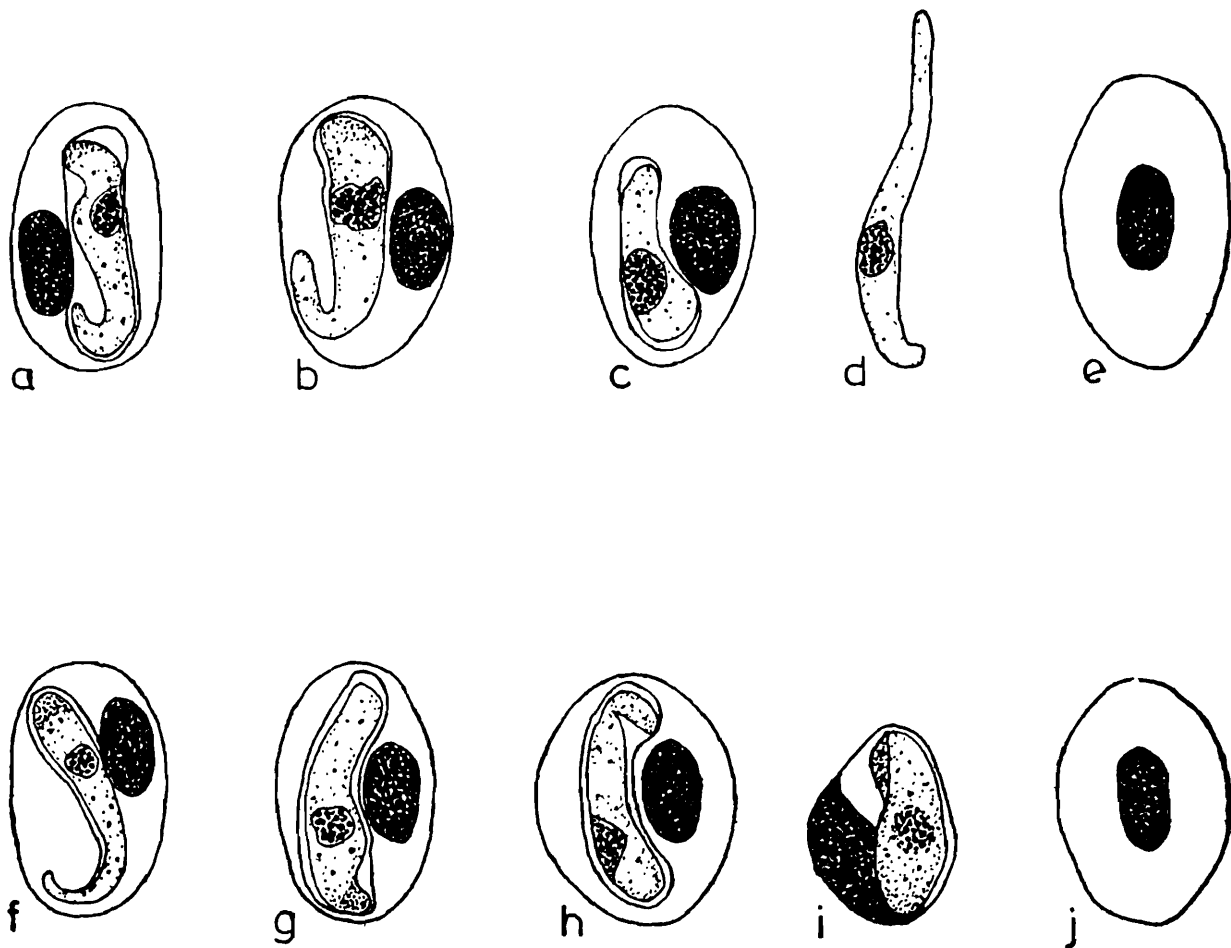


FIG. 3. HAEMOGREGARINA NUCLEOBISECANS.

10 μm

Fig. 3. Camera lucida drawings of the erythrocytic stages of *Haemogregarina nucleobisecans* from two anuran hosts.

a—d, the gametocyte stages of *H. nucleobisecans* in *Bufo melanostictus*. a & b, the elongate mature forms; c, the young form; d, the free gametocyte; e, the uninfected erythrocyte; f—i, the elongate mature gametocyte stages of *H. nucleobisecans* in *Bufo andersoni*; j, the uninfected erythrocyte.

Sometimes double infections within a single erythrocyte were also noted but not so common.

2) Intermediate and young form (Fig. 3c): Intermediate forms measure $10.5 \times 3.0 \mu\text{m}$ and were comparatively rare in the peripheral circulation. Cytoplasm stained light blue with fewer metachromatic granules. The nucleus is the aggregation of chromatin materials at the centre of the parasite. Young forms measure $5.2 \times 2.5 \mu\text{m}$; both the ends rounded, capsule-like, band shaped nucleus at the centre; cytoplasm stained deep blue with very few metachromatic granules.

3) Extra-corporal free form (Fig. 3d) : These were found in the peripheral blood in considerable number. They are elongated, with one end rounded and the opposite end being attenuated ; measuring $21.72 \mu\text{m}$ (0.19) by $2.84 \mu\text{m}$ (0.63) with an average area of $24.8 \mu\text{m}^2$ (1.36). The cytoplasm is bluish with metachromatic granules evenly distributed. The nucleus is a compact rounded mass stained purple with Giemsa and situated a little below towards the anterior end ; measuring $3.2 \mu\text{m}$ (0.24) by $2.28 \mu\text{m}$ (0.23) with an area $4.96 \mu\text{m}^2$ (0.85). Most extracorporal free forms were found excapsulated.

Effect of the parasite on the host cell : Elongate forms caused a marked hypertrophy of the infected red blood corpuscles and their nuclei. As a result of which the host cell became enlarged and the host cell nucleus is peripherally displaced sometimes pushed apical in position in case of double infection (Plate III Fig. 1). $\text{NDR}=0.17$ (0.05). Host cell hypertrophied in length (7.3%) and area (11.3%). Host cell nucleus also hypertrophied in area (1.7%) only.

Movement in fresh preparation : When a fresh sample of blood was mixed with saline citrate solution some parasites were found to excapsulate, escape from their host cell and move free in the fluid. The movement of free parasite was slow and typical of *Haemogregarina* type. The parasite advanced with its broader anterior end forwards, stopped for a while, bent sharply on itself, and then resumed its forward progress. Special organelle causing movement were not observed.

Uninfected erythrocytes : $N=15$. Cell $15.47 \mu\text{m}$ (0.79) by $8.55 \mu\text{m}$ (0.35) and $100.1 \mu\text{m}^2$ (10.69) in area. Cell nucleus $5.85 \mu\text{m}$ (0.22) by $2.72 \mu\text{m}$ (0.29) and $13.28 \mu\text{m}^2$ (1.66) in area. $\text{NDR}=0.97$ (0.04).

Schizogony (Plate III Figs. 7—10) : A thorough examination revealed some schizogonic stages or asexual multiplication in the liver parenchymatic cells. Other tissue sections like lungs, kidney, spleen etc. proved negative for the infection. Erythrocytic schizogony were not detected. A good number of schizonts both mature and immature forms were recorded either within the hepatic cells or in the liver parenchyma.

The early developmental stage of a schizont is an oblong or spherical uninucleated body occupying a cavity (Parasitophorous vacuole) (Fig. 7) within the liver cell. These bodies measure $4.5 \times 4.2 \mu\text{m}$. The spherical nucleus is $2.5 \mu\text{m}$ in diameter, surrounded by an areola. Cytoplasm granular, stains a good red colour and the nucleus is deep black when stained with Iron-alum haematoxylin and counter stained with eosin.

As the nucleus divides, 2 daughter nuclei occupy opposite poles of the schizont. Sometimes one nucleus was found to be elongated and this is probably due to the preparation for the next division. The latter is followed by successive division thus producing multinucleate stages (Plate III Figs. 8, 9). The daughter nuclei increase in number and arranged themselves peripherally. The multinucleate schizonts measure $15 \times 14 \mu\text{m}$.

The large mature schizont (Plate III Fig. 10) measures $20 \times 13 \mu\text{m}$ and contains remarkably larger number of merozoites. The merozoites are relatively slender and curved body measuring $4.5 \times 1.5 \mu\text{m}$ with both the ends rounded or sometimes one end attenuated. A more or less spherical nucleus is situated at the centre of the merozoite and stains deep black with Iron-alum haematoxylin stain. Intermediate stages between the early form and the mature schizont generally follow the same pattern described for development of small schizont.

Remarks: *Haemogregarina nucleobisecans* along with its hepatic schizogony was first reported from *B. melanostictus* by Shortt (1917) from Ambala (Punjab), Delhi and Cawnpore, India. Wenyon (1926) reported the same parasite as *Haemogregarina bisecans* from the same anuran host of India.

The present study of *H. nucleobisecans* well agrees with that of Shortt's description in all general respect. The bisecting nature of the hostcell nucleus, however, was not observed by the present authors. Shortt (1917) did not observe any movement of this parasite but the present authors noted a characteristic slow movement of extracorpuseular gametocytes in the citrated saline preparation of fresh blood. In the present study the same species has also been recorded from *Bufo andersoni*. *H. nucleobisecans* recorded from *B. andersoni* (Plate III Figs. 4—6) is a little smaller than that described from the type host (Table 4). It measures $13.8 \mu\text{m}$ (1.30) by $2.5 \mu\text{m}$ (0.44) with an average area of $24.97 \mu\text{m}^2$ (4.52). In *B. andersoni* some leucocytes were also found to be infected with parasites measuring $11.5 \mu\text{m}$ by $2.5 \mu\text{m}$.

A comparison of the mensural data of some related haemogregarina viz., *H. nucleobisecans*, *H. tunisiensis*, *H. aegyptia*, *H. boueti*, *H. pestanae*, *H. faiyemensis*, *H. moloensis* and *H. froilanoi* have been enumerated in the table 5. *H. nucleobisecans* resembles *H. boueti* but differs from it so far tissue schizogony is concerned.

Tissue schizogony of the parasite has been observed in both the hosts, *B. melanostictus* and *B. andersoni*. One type of schizogony with only one type of merozoite was noted. The characteristic features of the schizogonic stages in both the hosts are similar to those described by Shortt (1917).

4. *Haemogregarina perinucleophilum* Ray

(Figs. 4a—j; Plate IV Figs. 1—4)

1983b. *Haemogregarina perinucleophilum* Ray, *Rec. Zool. Surv. India*, 81 : 55.

Type-host : *Rana tigrina* Daudin.

Type-locality : Balitha, 5 Km. south west from Kotalpur, Bankura District, West Bengal, India.

Prevalence : Out of 164 examples of *Rana tigrina* examined 14 were found to be infected with this parasite. A minimum of 2% to a maximum of 8% of the R.B.C. counted were found to be infected with the parasite.

Description :

Gametocytes : Elongate mature form : N=15. Most of the mature parasites are elongated gregariniform having a broader head, attenuated thin body and a hook-like pointed tail. The parasites always encircled the host cell nucleus by its twisted body or sometimes superimposed with the host cell nucleus. In some cases they may push the host cell nucleus at one corner. The elongated form measures $11.1 \mu\text{m}$ (3.3) by $3.1 \mu\text{m}$ (0.76) with an average area of $25.7 \mu\text{m}^2$ (7.06). The larger one measures $18.5 \times 4.0 \mu\text{m}$ with an area of $37.5 \mu\text{m}^2$, occupying about 29.3% of total host cell-parasite complex. Cytoplasm densely granular, more thicker at the ends ; stained deep blue with Leishman's stain. Nucleus is not solid mass, oval or kidney-shaped, sometimes tubular, situated on the anterior half of the body ; stained purple or red with Leishman and Wright's stains. It measures $3.4 \mu\text{m}$ (0.72) by $2.2 \mu\text{m}$ (0.51) with an area of $6.0 \mu\text{m}^2$ (1.90) ; contains less compact chromatin granules.

No capsule was detected in this parasite. Tinctorial property and the structure of the nucleus may help to differentiate as microgametocytes and macrogametocytes. The microgametocytes having very clear cytoplasm without any granulation and nucleus is compact and band-like. Macrogametocytes having more dense cytoplasm and a compact nucleus.

Multiple infection of the host cell was not detected.

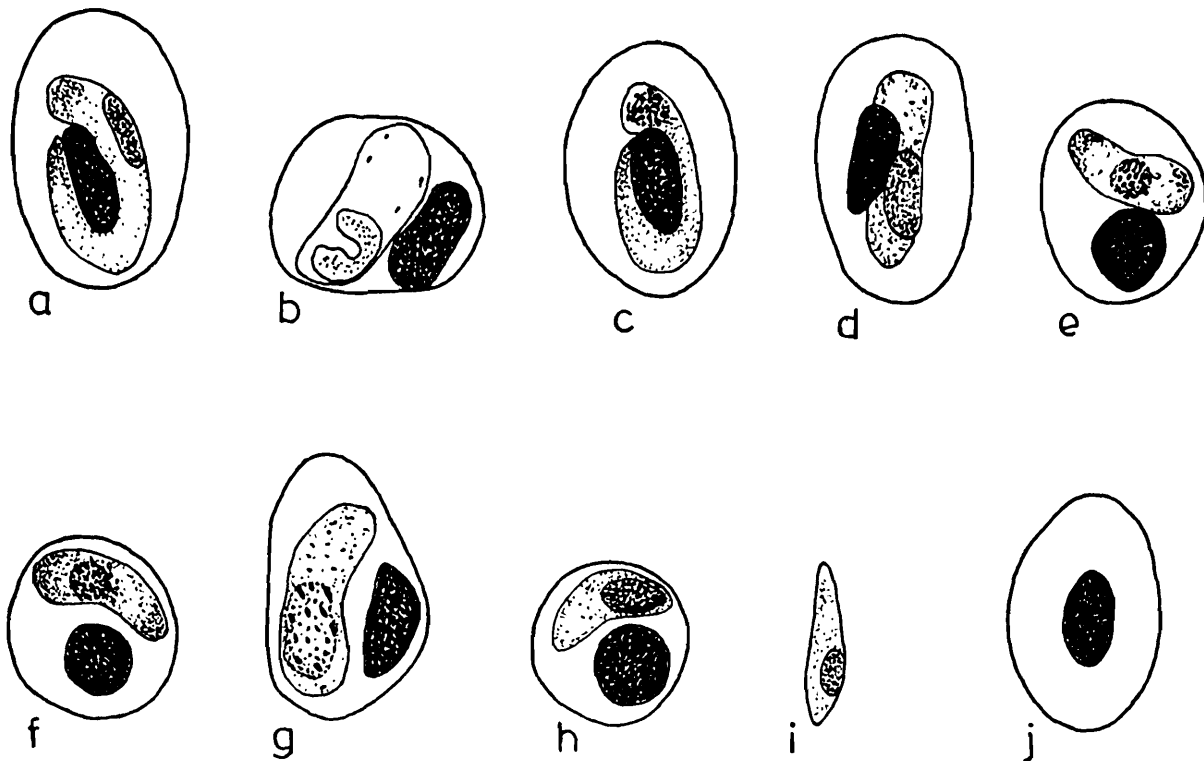


FIG. 4. HAEMOGREGARINA PERINUCLEOPHILUM.
SP. NOV.

10 μ m

Fig. 4. Camera lucida drawings of the erythrocytic stages of *Haemogregarina perinucleophilum* sp. nov. from *Rana tigrina*.

a—d, the elongate mature gametocytes ; a, the macrogametocyte ; b, the microgametocyte ; c & d, the mature gametocyte with apical nucleus and oval nucleus respectively ; e—g, the young broad forms of gametocytes, h, the young thin form ; i, the extra-corporeal free form ; j, the uninfected erythrocyte.

Young and Growing form : Young broad form : N=15. Elongated, both the ends rounded ; found mostly within the immature erythrocytes ; placed either perpendicular to the longitudinal axis of the erythrocytes or along its border. They measure $9.0 \mu\text{m} \times 3.2 \mu\text{m}$ with an area of $22.8 \mu\text{m}^2$. Cytoplasm is densely granular towards the ends and stained dark blue. Nucleus is the aggregation of some chromatin rods at the centre of the parasite crossing the entire width of the body ; stained deep red with Leishman. It measures $3.5 \mu\text{m} \times 2.0 \mu\text{m}$ with an area of $6.5 \mu\text{m}^2$.

Young thin forms: N=15. These are also curved elongated forms with both the ends more attenuated ; placed at the periphery of the erythrocytes. They measure $8.0 \mu\text{m} \times 2.5 \mu\text{m}$ with an area of $13.25 \mu\text{m}^2$; cytoplasm densely granular, dark blue in colour ; nucleus at one pole, measuring $4 \mu\text{m} \times 2 \mu\text{m}$ with an area of $4.5 \mu\text{m}^2$; stained purple red with Wright and Giemsa stains. Mostly immature erythrocytes are infected with this parasite.

Extracorpuscular free form : Small, elongated, gregariniform, comparatively fewer in number and are found in the plasma. Anterior end broader than the posterior which is more less pointed. They measure $9.6 \mu\text{m}$ (0.37) by $2.6 \mu\text{m}$ (0.37) with an area of $13.9 \mu\text{m}^2$ (0.95) ; cytoplasm homogeneous, granular ; stained deep blue with Leishman and Wright stains. The nucleus is round or ovoid, sometimes elongated, situated at the anterior half of the body towards the broader end. It consists of compact chromatin granules and stains purple with Leishman ; measuring $2.7 \mu\text{m}$ (0.22) by $1.5 \mu\text{m}$ (0.42) with an area about $2.0 \mu\text{m}^2$ (0.26).

Effect of the parasite on the host cell : The infected erythrocytes are little hypertrophied by the infection of larger elongated forms. But this hypertrophy with strong displacement of the nucleus occurs early in infection by younger forms. No double or multiple infections of a single cell were observed in the very large number of infected frogs examined. The host cell-nucleus became rounded and displaced either a little below the central position or sometimes at one corner of the host cell. $\text{NDR}=0.26$ (0.05). Host cell hypertrophied only in width (12.7%). No remarkable change in length and area was noted. Host cell nucleus hypertrophied in area (26.22%).

Movement in fresh preparation : When fresh blood mixed with citrated saline was observed, some parasites were seen to come out of their host cells. On release they exhibit a very slow gliding movement with their broad anterior ends projecting forward. No rhythmic undulating movement was noted neither any special organelle for movement was observed.

Uninfected erythrocyte : $N=15$. Cell $13.0 \mu\text{m}$ (1.5) by $8.6 \mu\text{m}$ (0.82) and $87.0 \mu\text{m}^2$ (11.25) in area. Cell nucleus $5.4 \mu\text{m}$ (0.56) by $2.5 \mu\text{m}$ (0.41) and $11.1 \mu\text{m}^2$ in area. $\text{NDR}=0.90$ (0.07).

Schizogony : Repeated examination revealed some schizogonic stages in the erythrocytes of the circulating blood. As the parasites enlarges the nucleus divides. Binucleated, quadrinucleated and octonucleated schizonts are of common occurrence. A mature schizont engulfs almost the whole erythrocyte.

The tissue schizogony has not been recorded so far.

Remarks: Ray (1983b) reported this parasite from *Rana tigrina*. *Haemogregarina perinucleophilum* can be distinguished from the other haemogregarines by the fact that the mature gametocyte 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 which is the typical character of the parasite and this parasite also differs from the same on the basis of morphometric parameters (Table 6). It can also be differentiated from *H. magna* by its characteristic encircling nature to its host cell nucleus, and by possessing densely granulated cytoplasm and also in the position and structure of the nucleus. In other morphological parameters too *H. perinucleophilum* exhibits remarkable differences with *H. magna* and *H. berestneffi* (Table 7) described from the same host genus.

5. ***Haemogregarina maculatus* sp. nov.**

(Figs. 5a—c ; Plate IV Figs. 5—8.)

Type-host : *Rhacophorus maculatus* (Gray)

Type-locality : Balitha, 5 Km. south west from Kotalpur, Baukura district, West Bengal, India.

Type-material : On slides

Holotype—Z.S.I., Reg. No. Pt. 1995

Paratype—Z.S.I., Reg. No. Pt. 1996

Coll. Dr. R. Ray

Description : The description is based on the parasites obtained from the blood of a tree frog, *Rhacophorus maculatus* collected during June—July, 1977.

Gametocytes : Elongate mature form (Figs. 5a—d ; Plate IV Figs. 5—7, Tables 8, 9) : N=15. These forms are considered as gametocytes. Intra-corporal, elongated to club-shaped, having broader anterior end with a neck-like constriction and on the attenuated end there is a plug like structure. Cytoplasm is granular but not homogeneous ; granules are thicker on the two poles thereby giving rise the appearance of a cap-like structure and always stained deep blue with Leishman and Wright stains. The middle portion of the parasite is very thin, more or less non-granular and either stained faint or remained unstained in some parasites. This elongate forms measure $16.5 \mu\text{m}$ (0.68) by $3.3 \mu\text{m}$ (0.61) with an area of $40.5 \mu\text{m}^2$ (6.09), occupying about 38.4% of the total host cell-parasite complex. The nucleus is rhomboidal or rounded, stains red with Giemsa and Leishman, always situated on the broader anterior half of the parasite. It measures $2.4 \mu\text{m}$ (0.20) by $2.1 \mu\text{m}$ (0.19) with an average area of $4.4 \mu\text{m}^2$ (0.58) and contains compact chromatin granules. The large parasite gains full length of the

erythrocyte and is situated on one side of the host cell nucleus. The intra-erythrocytic gametocytes do not possess any capsule.

Differences in parasite's staining responses and the structure of the nucleus suggest its possible sexual dimorphism. The male or microgametocytes are comparatively light stained i.e. clear cytoplasm and the chromatin materials of the nucleus are less compact. The female or macrogametocytes

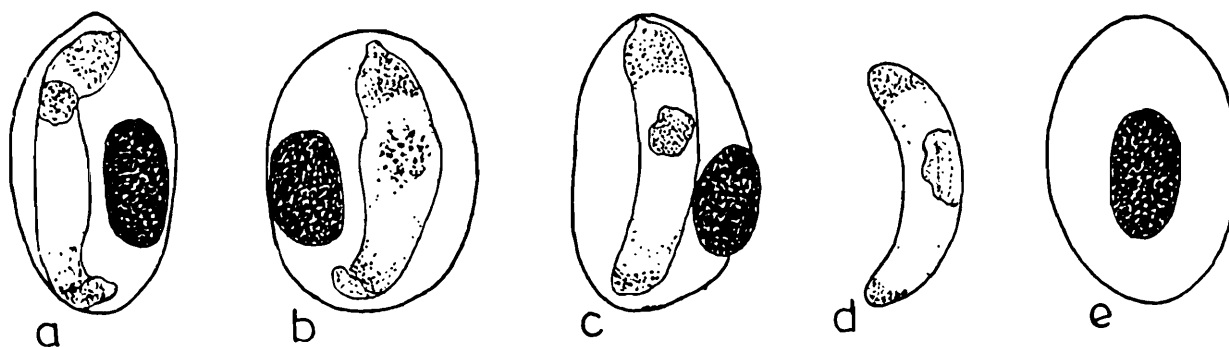


FIG. 5. HAEMOGREGARINA MACULATUS.
SP. NOV.

10 μm

Fig. 5. Camera lucida drawings of the erythrocytic stages of *Haemogregarina maculatus* sp. nov. from *Rhacophorus maculatus*.

a—d, the elongate mature gametocytes. a & c, the macrogametocyte ; b, the microgametocyte ; d, the extra-corpuseular free gametocyte ; e, the uninfected erythrocyte.

are deep stained and having a rounded compact prominent nucleus situated towards the broader anterior end of the parasites. Such difference in staining responses may be visible and distinguishable in single optical field.

Intermediate or young forms were not encountered in the slides prepared during the study.

Extracorpuseular free form (Fig. 5d): Fewer in number and always found as free plasma parasite. These are elongated, either straight or curved parasites with one end more broader than the other. They measure $15.2 \mu\text{m}$ (0.92) by $2.95 \mu\text{m}$ (0.15) with an area of $29.73 \mu\text{m}^2$ (1.92). Cytoplasm is finely granular, non-homogeneous, granules are thick at the poles producing a cap-like structure ; stained blue with Leishman and Wright. They do not have any beak-like structure which have been observed in the intracorpuseular forms. The nucleus is tubular, compact, situated at the centre or towards the broader end ; stained purple to red with Leishman and Giemsa. It measures $4.0 \mu\text{m}$ (0.05) by $1.74 \mu\text{m}$ (0.17) with an area of $5.57 \mu\text{m}^2$ (0.06).

Effect of the parasite on the host cell : The infected red blood cells underwent slight linear hypotrophy. The mesural data shows that slight elongation of the host cell was partly compensated by a corresponding

decrease in width. The convex side of the parasite (Fig. 5c) faced the host cell nucleus more frequently than did the concave side (Fig. 5a). The host cell nucleus is displaced to the periphery or lies in any position but never apically. Sometimes double infection (Fig. 8) of the parasite may also be noted when the host cell became very much distorted and the cell nucleus pushed at one corner. $NDR=0.23$ (0.15). Host cell nucleus atrophied in area (3.39%).

Movement in fresh preparation: In citrated saline preparation the parasite did not appear to be very active. A slight gliding movement was observed.

Uninfected erythrocytes: $N=15$. Cell $14.75 \mu\text{m}$ (1.10) by $10.0 \mu\text{m}$ (0.20) and $102.1 \mu\text{m}^2$ (5.56) in area. Cell nucleus $6.16 \mu\text{m}$ (0.37) by $3.66 \mu\text{m}$ (0.47) and $17.06 \mu\text{m}^2$ (2.03) in area. $NDR=0.96$ (0.03).

Schizogony and vector unknown

Prevalence: Out of 177 examples of *Rhacophorus maculatus* Gray examined from different parts of India only 11 were found to be infected with this parasite. A minimum of 1% to a maximum of 5% of the total R.B.C. counted were positive for the parasites.

Remarks: Haemogregarines and parasites of the genera *Haemoproteus* and *Leucocytozoon* are mostly known and identified only by their gametocytes. Mohammed and Mansour (1959) clearly stated that, haemogregarine gametocytes are better criteria for specific rather than generic identification. This situation is due to the fact that the classification of the group into genera (and families) is based on the sporogenous cycle, which is known in only a few species. Therefore, the authors intend to relate the parasite communicated in this monograph as belonging to the genus *Haemogregarina sensu latu*.

On the other hand gametocytes often show such characteristics that help their easy identity. Differences in the confirmity of the sexual forms in the blood may reflect other and probably more, pronounced variations in the unknown parts of the developmental cycle of the parasites.

A perusal of literature on amphibian haematozoa, so far as the haemogregarines are concerned, indicates that a particular species of *Haemogregarina* does not show its distribution range beyond a particular host family.

From this point of view the authors claim to report for the first time from India any haemogregarine parasite from the family Rhacophoridae.

Haemogregarina maculatus sp. nov. is quite different from previously described haemogregarine species from toads and frogs including the two (*H. magna* and *H. berestneffi*) from the Indian anuran hosts belonging to the family Ranidae. Differences in size, nuclear structure and position, cytoplasm, staining responses and presence of beak-like structure are particular important features for diagnosis and were taken into consideration.

The present parasite differs from *H. berestneffi* in not having any capsule and also differs from *H. magna* by its structure and position of the nucleus and staining property. It also begs to deviate from them by having beak-like projection at the anterior end and plug-like structure at the posterior end in which it is unique.

Haemogregarina maculatus sp. nov. seems to proximate with *H. theileri* reported from *Rana angolensis* and *Haemogregarina* sp. from *R. wittei* of Africa (Ball, 1967) by having similar cap-like structure with small beak or nipple at the broader end. But the mensural data of those species or other characters are obviously different from the present one (Table 9).

Thus considering strong familial specificity among the Haemogregariniidae as regards their host and for the characteristic morphologies displayed by the present parasite, the name *Haemogregarina maculatus* sp. nov. is proposed to its new specific identity.

6. *Haemogregarina pattoni* sp. nov.

(Figs. 6a—e ; Plate IV Figs. 9—13)

Type-host : *Rana hexadactyla* Lesson

Type-locality : Midnapore, West Bengal, India.

Type-material : On slides

Holotype—Z. S. I., Reg. No. Pt. 1997

Paratype—Z. S. I., Reg. No. Pt. 1998

Coll. Dr. R. Ray

Description : The following description is based on the haemogregarine obtained from the Indian green frog, *Rana hexadactyla*.

Gametocytes : 1) Elongate mature form (Fig. 6a, Table 10) N=15. All the erythrocytic forms are considered as gametocytes. Gamonts are elongated with anterior broader rounded end and posterior attenuated end. They usually occupy half of the R.B.C. complex and folded upon themselves according to their structure and orientation in the host cells. They

measure $16 \mu\text{m}$ by $3.8 \mu\text{m}$ with an area of $29.0 \mu\text{m}^2$; occupying about 28.7% of total host cell-parasite complex. Cytoplasm densely granular in the middle near the nucleus but lighter towards the poles; stained blue with Leishman and Wright stains. Nucleus is oval to round; situated almost in the middle of the parasite covering the whole width of the body. It measures $3.5 \mu\text{m}$ by $2.5 \mu\text{m}$ with an area of $7.0 \mu\text{m}^2$. Chromatin granules are compactly arranged and no clear karyosome is visible.

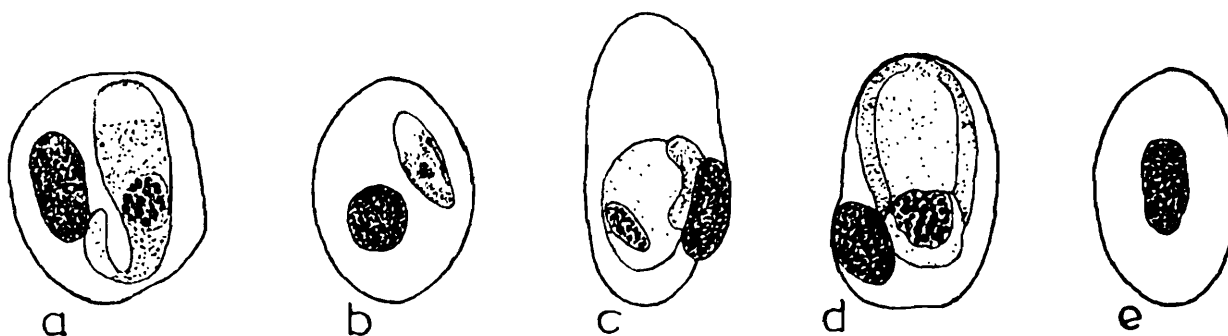


FIG. 6. HAEMOGREGARINA PATTONI
SP. NOV.

10 μm

Fig. 6. Camera lucida drawings of the erythrocytic stages of *Haemogregarina pattoni* sp. nov. from *Rana hexadactyla*.

a, the elongate mature gametocyte; b, the young thin form; c, the young broad form with single capsular band; d, the mature capped form; e, the uninfected erythrocyte.

2) Capped form (Plate IV. Figs. 9—13, Table 10): N=15. The most frequent and characteristic forms of this parasite are the capped forms which are found abundantly in the peripheral blood. These are elongated with both the ends rounded or sometimes attenuated, measuring $9.7 \mu\text{m}$ (2.76) by $4.93 \mu\text{m}$ (1.29) with an area of $33.05 \mu\text{m}^2$ (8.30), occupying 36.4% of the total host cell-parasite complex. They are situated either on the longitudinal axis or on the transverse axis or anywhere within the red blood corpuscles. The parasites are surrounded by a very thin non-visible capsule or outer covering. Cytoplasm stains light blue in colour, nongranulated. The most interesting structure of these parasites is the capsular band which stains dull red with Giemsa, Leishman and Wright and appears to fit over the parasite. These are situated either on the periphery (Fig. 9) of the parasite as elongated structure or on any one side (Fig. 11) or sometimes it passes over the parasite obliquely (Fig. 10). Capsule with different developmental phases have also been observed. These measure $6.0 \mu\text{m}$ (2.02) by $1.25 \mu\text{m}$ (0.35) with an area of $5.15 \mu\text{m}^2$ (2.25). When unstained, the cap is well defined, translucent area, while in lightly stained specimens a number of

red, coarse, rounded granules appear. While, during progressive staining, the granules increase in number and eventually appear as dull pink-staining mass.

The cytoplasm of these parasites is clear, nongranular ; sometimes a few red staining granules appear surrounding the nucleus ; stained light blue with Leishman. The nucleus is rhomboidal, angular or rounded and situated either in the middle or a little below towards the posterior end.

In the capped form, the nuclear structure and cytoplasmic reaction clearly differentiate the male gametocytes from the female one. The male gametocytes are always light-stained and nucleus is in the form of reticulum, distributed throughout the cytoplasm. Whereas the female gametocytes display darker cytoplasm and compact flower-like nucleus.

3) Young and Growing forms : *Thin form*—N=15. These are thin with both the ends tapering i.e. spindle shaped (Fig. 6b). The youngest one measures $6\ \mu\text{m}$ by $2\ \mu\text{m}$ with an area of $8.7\ \mu\text{m}^2$. Cytoplasm nongranular, stained light blue with Leishman. The capsular band is present at the periphery which stained dull red. The round nucleus measures $1.0\ \mu\text{m}$ in diameter and lies at the middle of the parasites. The nuclear membrane is not visible and chromatin granules are loosely arranged. These forms are found abundantly in the immature erythrocytes.

Broad form—N=15. The young broad forms are oval to rounded in structure measuring $7\ \mu\text{m}$ by $5\ \mu\text{m}$ with an area about $26.5\ \mu\text{m}^2$; situated on one side of the R.B.C., pushing the host cell-nucleus at the periphery ; cytoplasm is nongranular and clear. The nucleus is oval to elongate in shape measuring $2.5\ \mu\text{m}$ by $1.5\ \mu\text{m}$ with an area of $3.0\ \mu\text{m}^2$; situated centrally or towards one pole of the parasite. The capsular band is elongated, well visible on the periphery of the parasite close to the host cell nucleus.

Extracorpuseular free form (Plate IV Fig. 13) : N=15. These forms are very few in number and are observed free in the plasma. They may have natural exit from the corpuscles by means of natural way or may be freed by mechanical damage. These free parasites always having an oval capsule and in this stage the capsule is better demonstrable as a transparent, faint, pink membrane surrounding the parasite (Fig. 13). The parasite is elongated with both the ends rounded. They measure $12.5\ \mu\text{m}$ by $3.5\ \mu\text{m}$ with an area of $30.2\ \mu\text{m}^2$, cytoplasm lightly blue when stained with Leishman. Nucleus is rounded, compact structure, measuring $3.5\ \mu\text{m}$ by $3.0\ \mu\text{m}$ with an area of $8.7\ \mu\text{m}^2$, situated on the posterior half of the body.

Effect of the parasite on the host cell : Red blood corpuscles infected with capped forms are invariably hypertrophied. Moreover, the host cell nucleus is often pushed aside (Fig. 6c) or strongly displaced obliquely near one of the poles of the cell (Fig. 6d). Characteristically, infected corpuscles are folded, wrinkled and disfigured, appearing as a collapsed sac. This hypertrophy with strong displacement of the nucleus occurs early in infection by younger forms. Double or multiple infection in a single cell was not observed in the large number of infected frogs examined. $NDR=0.21$ (0.03). Host cell hypertrophied in length (17.5%), in width (13.0%) and area (44.2%). The host cell nucleus hypertrophied in area (12.3%).

Movement in fresh preparation : In the fresh preparation of blood with citrated saline, some capsulated parasites were found to come out of the R.B.C. They showed feeble movement within the capsule which cannot be correlated with the gliding type of movement.

Uninfected erythrocytes : $N=15$. Cell $11.1 \mu\text{m}$ (0.14) by $7.7 \mu\text{m}$ (0.78) and $62.8 \mu\text{m}^2$ (3.08) in area. Cell nucleus $4.4 \mu\text{m}$ (0.52) by $2.74 \mu\text{m}$ (4.61) and $9.7 \mu\text{m}^2$ (1.30) in area. $NDR=0.96$ (0.06).

Schizogony and vector unknown.

Prevalence : Out of 180 examples of *Rana hexadactyla* Lesson examined, 24 were found to be infected with this parasite. A minimum of 3% to a maximum of 17% of the total R.B.C. counted were found positive for the parasite.

Remarks : Patton in 1908 for the first time recorded a haemogregarine from *Rana hexadactyla* of Madras. But he did not mention the species encountered. He also mentioned that he had the opportunity of studying no less than five haemogregarines in *R. tigrina* and *R. hexadactyla* from Madras.

A perusal of literature suggests that the present parasite does not resemble with any other haemogregarine being reported so far from India. The most important and interesting structure of the parasite presented here is the presence of a dull coloured "cap" or a unique 'band-like' structure.

The encapsulated "capped" gametocytes of the present parasite differs from *H. berestneffi* and *H. magna* in morphometric measurements and in the cap-like structure. The parasite under consideration is also comparatively smaller in size than the other two.

Parasite and reference	Gametocyte		Nucleus	
	Length in μm	Width in μm	Length in μm	Width in μm
<i>H. berestneffi</i> Castellani and Willey, 1905 (After Bhatia, 1938)	26—28	4—5.5	—	—
<i>H. berestneffi</i> (from the present study)	16.5	2.57	2.85	2.19
<i>H. magna</i> Grassi and Feletti, 1891 (Reproduced from Levine and Nye, 1977)	18.0	6	4	4
<i>H. magna</i> (from the present study)	12.4	4.06	4.4	3.3
<i>H. pattoni</i> sp. nov.	16.0	3.8	3.5	2.5

The rapid gliding movement of the free extracorporeal forms of *H. berestneffi* and *H. magna* was not observed in this case. The movement is very very sluggish and when freed, the parasite always displays a broad oval thin capsule.

From the point of view of "cap" or band pattern the present parasite may be brought into proximity with *H. aegyptia* Mohammed and Mansour and *H. tunisiensis* Nicolle, both described from *Bufo regularis*, an African anuran host. Morphometrically the Indian haemogregarine falls short in measurements to that of the African species, specially in cytoplasm/nucleus ratio. The band pattern in *H. aegyptia* and *H. tunisiensis* is egg-shaped in former and regularly oval around the parasite in the latter, whereas it is more or less parenthetically extended along the lateral border in case of the Indian haemogregarine described from *R. hexadactyla*.

Parasite and reference	"Capped" Parasite		Nucleus	
	Length in μm	Breadth in μm	Length in μm	Breadth in μm
<i>H. pattoni</i> sp. nov.	9.70	4.93	3.42	2.50
<i>H. aegyptia</i> Mohammed and Mansour, 1963	14	7.3	3.7	2.9
<i>H. tunisiensis</i> Nicolle, 1904	12—15	8	—	—

Haemogregarina aurorae Lehmann, 1960 reported from *Rana a. aurora*, resembles to some extent the parasite under discussion in its extracorporeal capsule design which becomes evident when the gametocytes are freed in the blood plasma, but the two parasites differ in other morphological details.

From the foregoing meticulously reviewed account it becomes apparent that the haemogregarine from *R. hexadactyla* presented in this treatise obviously claims its separate and independent identity, and to the present

authors, the parasite seems to be new to science. So the name *Haemogregarina pattoni* sp. nov. is proposed for the parasite in the memory of Dr. W. S. Patton who is credited first to record haemogregarine from *R. hexadactyla* in India but refrained from awarding a species name for it for unknown reasons.

7. ***Haemogregarina kaloulae* sp. nov.**

(Figs. 7a—e ; Plate V Figs. 1—8)

Type-host : *Kaloula pulchra taprobanica* Parker.

Type-locality : Bishnupur, Bankura District, West Bengal, India.

Type-material : On slides.

Holotype : Z. S. I., Reg. No. Pt. 1999

Paratype : Z. S. I., Reg. No. Pt. 2000

Coll. Dr. R. Ray

Description : The parasite reported herein was obtained from microhylid Ceylon Kaloula, *Kaloula pulchra taprobanica* Parker collected from Bishnupur, Bankura district, West Bengal, India. The erythrocytic gametocytes, extra-corpuseular free form and tissue schizogony are described as follows :

Gametocytes : (Figs. 8a—d, Plate V Figs. 1—4, Table 11) :

Elongate mature form : N=15. These are elongated with both the ends rounded or sometimes posterior end is attenuated to form hook-like structure (Fig. 8, a & Plate V Fig. 2). They are usually situated at one half of the erythrocytes along with its whole longitudinal axis facing the concave border of the parasite towards the convex side of the host cell-nucleus. This position may be changed according to the structure and posture of the parasite as well as the host cell. Cytoplasm is granular, more dense towards the poles, thereby giving rise to cap-like structure measuring $3.75 \mu\text{m}$ (0.43) by $2.87 \mu\text{m}$ (0.59) and area of $8.75 \mu\text{m}^2$ (1.92), which are very conspicuous characteristics of the parasite ; it stained pink colour with Leishman and Giemsa. The elongated mature forms measure $16.02 \mu\text{m}$ (1.22) by $3.65 \mu\text{m}$ (0.51) with an area of $43.22 \mu\text{m}^2$ (6.66), occupying about 38.2% of the total host cell-parasite complex. The nucleus is rounded oval, triangular or band-shaped, usually situated at the anterior end and stained purple red with Leishman which almost covers the whole width of the parasite ; measuring $3.9 \mu\text{m}$ (0.91) by $2.06 \mu\text{m}$ (0.38) with a mean area of $6.12 \mu\text{m}^2$ (1.68). The chromatin materials are compact. Definite nucleus membrane is visible.

The parasite is enclosed within a closely fitted dark red stained capsule measuring $16.5 \mu\text{m}$ by $3.75 \mu\text{m}$ with an area of $44.5 \mu\text{m}^2$. Sometimes this capsule may not be visible.

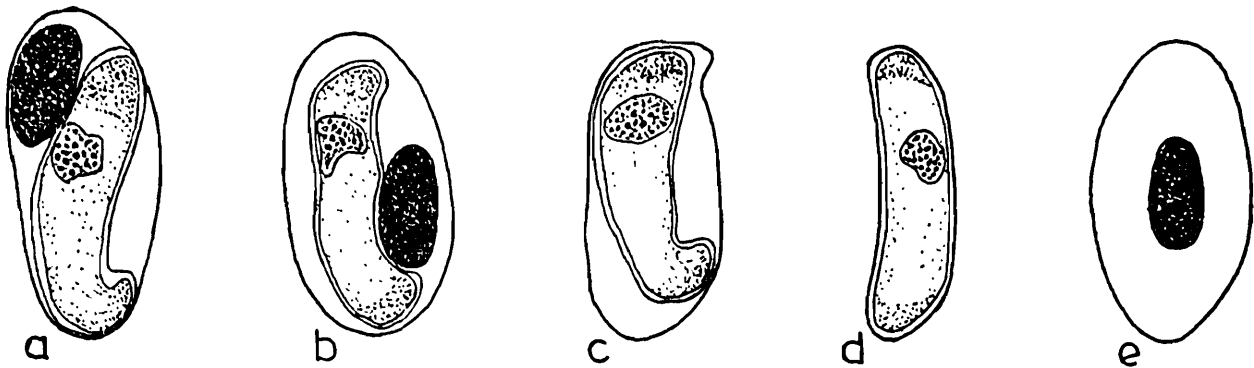


FIG. 7. HAEMOGREGARINA KALOULAE
SP. NOV.

10 μm

Fig. 7. Camera lucida drawings of the erythrocytic stages of *Haemogregarina Kaloulae* sp. nov. from *Kaloula pulchra taprobanica*.

a—d, the gametocyte stage. a & b, the elongate, capsulated mature forms ; c, the enucleated R.B.C. containing gametocyte ; d, the extra-corpuseular free gametocyte ; e, the uninfected erythrocyte.

No intermediate or growing forms were observed.

Extracorpuseular free form (Fig. 7b, Plate V Fig. 6) : N=15. These are also elongated parasite with both the ends rounded, found in the plasma. They measure $17.03 \mu\text{m}$ (0.28) by $3.16 \mu\text{m}$ (0.22) with an area of $45.69 \mu\text{m}^2$ (2.29). Cytoplasm granular, nonvacuolated, stained dark blue with Leishman and Wright. The extracorpuseular free forms always bearing a thin closely fitted capsule. The nucleus is rounded to oval, stained purple ; situated centrally or a little below ; measuring $3.37 \mu\text{m}$ (0.37) by $2.06 \mu\text{m}$ (0.14) with an area of $5.13 \mu\text{m}^2$ (0.62).

Effect of the parasite on the host cell : Erythrocytes containing large capped gametocytes are very much hypertrophied. Characteristically infected red blood corpuscles are wrinkled, disfigured and sometimes appeared as collapsed sac. This host cell became dehaemoglobinized and greatly distorted with one or both the poles sharply pointed. Double or multiple infection is very rare. The host cell nucleus is also hypertrophied and they are pushed apart from the centre into the periphery or sometimes at one pole of the erythrocytes. It is evident that sometimes the host cell is completely filled up by the parasite and thereby the cell nucleus is completely being rejected out (Fig. 7c). NDR=0.14 (0.01). Host cell

hypertrophied in length (14.5%) and in area (7.5%). The host cell nucleus hypertrophied in area (20.7%).

Movement in fresh preparation : The fresh preparation of blood mixed with anticoagulant (citrated saline soln.) revealed a beautiful gliding movement of the parasite inside the capsule. Movement involved changes of shape, bending, wriggling and uncoiling of the parasites. Sometimes the parasites were found to be folded centrally with both the apices.

Uninfected erythrocytes : N=15. Cell $15.65 \mu\text{m}$ (0.80) by $8.92 \mu\text{m}$ (0.49) and $95.82 \mu\text{m}^2$ (6.08) in area. Cell nucleus $6.4 \mu\text{m}$ (0.34) by $2.3 \mu\text{m}$ (0.47) and $12.2 \mu\text{m}^2$ (1.77) in area. NDR=0.96 (0.05).

Schizogony (Plate V Figs. 7, 8) : Asexual multiplication or schizogony was observed in the erythrocytes where the nucleus of the parasite undergoes cleavage forming binucleate schizonts.

The tissue schizogony inside the liver parenchyma were noted in a number of cases. The schizonts, both uninucleate and multinucleate, were located in between the polyhedral liver parenchymatic cells very near the central vein. The schizonts are rounded, triangular or elongated in structure containing very small nuclei of about $1.0 \mu\text{m}$ in diameter and 20-35 in number. The multinucleated rounded schizonts (Plate V Fig. 7) measure 12-16 μm in diameter. The nuclei stained deep black and the cytoplasm light yellowish blue with Iron-alum haematoxylin stain. Sometimes the mature schizont burst and released the merozoites which are very minute ovoid structure (Plate V Fig. 8) distributed throughout the parenchyma cells. The parasitophorous vacuole is not well marked as it is closely packed with the parenchyma cells.

Prevalence : Out of 204 examples of *Kaloula pulchra taprobanica* Parker examined from different districts of West Bengal, 17 were found to be infected with the present parasite. The intensity of infection is a minimum of 4.5% and a maximum of 8% of the total R.B.C. counted.

Remarks : A perusal of literature revealed that there is no report of *Haemogregarina* infection from the microhylid frog, *Kaloula pulchra taprobanica*.

The haemogregarines of amphibia may be considered specific at the host family level as stated earlier. The host frog is a member of the family Microhylidae and the species, *Kaloula pulchra taprobanica* is endemic one. The frogs belonging to the family Ranidae harbour two haemogregarine

species viz., *H. berestneffi* Castellani & Willey, 1905 and *H. magna* (Grassi & Feletti, 1891) in India.

Haemogregarina kaloulae sp. nov. closely resembles *H. berestneffi*, a parasite of *Rana tigrina* and other Ranid frogs. The mature gametocytes of *H. berestneffi* possess similar capsule and nuclear structure like the species described in the present treatise. However, the present species differs from the former in having definite polar caps on both the ends and being broader in width.

The present parasite also resembles *H. theileri* Laveran 1905, reported from *Rana angolensis* of East Africa by possessing similar cap-like structure and closely fitted sheath in the mature gametocytes. But the present haemogregarine species is smaller than *H. theileri* (18.9 X 6.9 μ m). It also differs in not having the typical nipple-like structure at the anterior end which is the characteristic feature of the free gametocytes of *H. theileri*.

The examination of the type of schizonts in the liver parenchyma of *K. p. taprobanica* marks another point of interest in the present parasite. The schizonts are small, rounded, triangular or elongated in shape and always found by the side of the large central vein of the liver, which seems to be the main site of schizogony activity. From this fact it may be assumed that the merozoites while passing through the central vein choose their preferred site of multiplication by the side of it, and in the cells of the liver. Erythrocytic schizogony though scanty in its occurrence, is also an added chapter in the vertebrate cycle of the parasite.

As there was no earlier record of *Haemogregarina* from the host *K. p. taprobanica* and considering the strong familial specificity the present parasite is considered as new to science and is named *Haemogregarina kaloulae* sp. nov. after its host's name,

8. ***Haemogregarina andamanensis*** sp. nov.
(Figs. 8a—j; Plate V Figs. 9—11)

Type-host : *Rana cyanophlyctis* Schneider.

Type-locality : Wright Myo, Andaman Islands, India.

Type-material : On slides.

Holotype : Z. S. I., Reg. No. Pt. 2001

Paratype : Z. S. I., Reg. No. Pt. 2002

Coll. S. S. Saha.

Description : The present description is based on the parasite obtained from Indian skipping frog, *Rana cyanophlyctis*. The following stages were found in the blood of the hosts.

Gametocytes : Elongate mature form (Figs. 8a—j ; Plate V Figs. 9—11, Table 12) : N=15. Elongated with both the ends rounded, occupies almost completely the whole length of the erythrocyte. Their concave side faces towards the convex side of the host cell-nucleus or do not follow any definite pattern. The various forms taken by the parasite probably depend on its length in relation to the size of the host cell. They measure $14.55 \mu\text{m}$ (1.76) by $3.45 \mu\text{m}$ (0.56) with an area of $45.69 \mu\text{m}^2$ (5.09) ; occupying about 45% of the total host cell-parasite complex. Cytoplasm is very clear, homogeneous, nongranulated, stained light blue colour in Giemsa stain. The nucleus is central or subcentral in position usually rounded, measuring $2.05 \mu\text{m}$ (0.41) by $1.7 \mu\text{m}$ (0.24) with an area of $2.85 \mu\text{m}^2$ (0.80). Nucleus stained purple red with Giemsa, consisting of a few chromatin granules which are loosely arranged. No clear nuclear membrane was visible and no karyosome could be detected. Parasites also do not exhibit any sheath or capsule. Presence of large number of elongated intra-erythrocytic parasites in the peripheral blood represent the chronic phase of the infection.

No young or growing forms were detected.

Extracorpuseular free form (Fig. 8j) : N=15. These are also elongated with both the ends rounded. The posterior end is a little attenuated but never pointed. They measure $15.6 \mu\text{m}$ (0.36) by $4.25 \mu\text{m}$ (0.25) with an area of $54.76 \mu\text{m}^2$ (1.29). The cytoplasm is clear, homogeneous, nongranular ; stained light blue with Giemsa Stain. The nucleus is rounded, central and subcentral in position, measuring $1.71 \mu\text{m}$ (0.22) in diameter, consisting of a few loosely arranged chromatin granules ; stained purple with Giemsa ; nuclear membrane not visible.

Effect of the parasite on the host cell : Parasitized red blood cells are conspicuously hypertrophied. Sometimes they may be folded, wrinkled, and disfigured, appearing as a collapsed sac.

The host cell nucleus is usually markedly displaced to one side ; the posterior end of the parasite may be bent away from or more frequently towards the host cell nucleus. The host cell is then characteristically straightened on one side by the enlarged parasite. (Plate V Fig. 9). Less frequently the nucleus may be displaced by the parasite towards the apex of the cell (Fig. 8c).

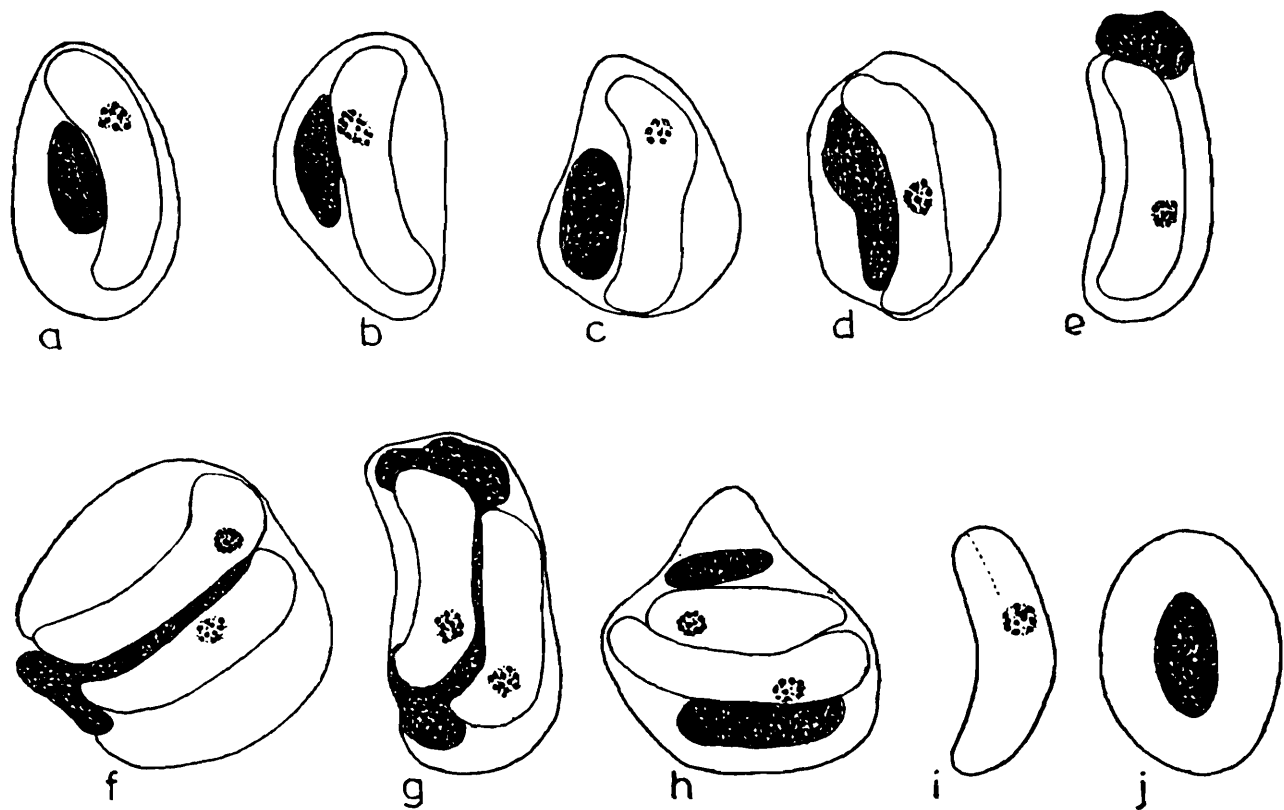


FIG. 8. HAEMOGREGARINA ANDAMANENSIS.
SP. NOV.

10 μm

Fig. 8. Camera lucida drawings of the erythrocytic stages of *Haemogregarina andamanensis* sp. nov. from *Rana cyanophlyctis*.

a—i, the gametocyte stages. a—d, the elongate mature gametocyte; e, the gametocyte pushes the host cell nucleus towards the apical position of the host cell; f & g, the double infection with hypertrophied host cell and host cell nucleus; h, the double infected hypertrophied host cell where the host cell nucleus bisected into two parts; i, the extra-corporeal free gametocyte; j, the uninfected erythrocyte.

Another remarkable feature is that the host cell nucleus is frequently divided into 2 more or less equal or unequal fragments. The fragments may lie close to each other or be widely separated. This phenomenon is of special interest and commonly found in case of double or triple infection only. In case of double infection the host cell becomes very much distorted or disfigured (Fig. 8f, g) and the two cohabitants lie side by side along the length of the much distended corpuscle. Fragmentation of the host cell nucleus started with hypertrophy and elongation of the nucleus, followed by a constriction and they then completely separated and oriented on the two poles of the parasitized erythrocytes (Fig. 8i). NDR=0.4 (0.27). Host cell hypertrophied in length (4.8%) and in area (15%). The host cell nucleus hypertrophied in area (33.1%).

Movements in fresh preparation: Freshly drawn blood mixed with citrated saline solution revealed a beautiful gliding movement of the parasites when they come out of the erythrocytes. They move forward with the anterior end and this movement involves bending and twisting of the parasites. No special organellae for movement was detected.

Uninfected erythrocytes: N=15. Cell $15.22 \mu\text{m}$ (0.84) by $9.97 \mu\text{m}$ (0.39) and $109.63 \mu\text{m}^2$ (9.82) in area. Cell nucleus $5.95 \mu\text{m}$ (0.85) by $3.45 \mu\text{m}$ (0.51) and $12.82 \mu\text{m}^2$ (3.82) in area. NDR=1.01 (0.1).

Schizogony and vector unknown.

Prevalence: Out of 450 examples of *Rana cyanophlyctis* examined from different parts of India, only 3 were found to be infected with this parasite. A minimum of 2% to a maximum of 8% of the total R.B.C. counted were positive for the parasite.

Remarks: A perusal of the literature on the amphibian haematozoa revealed that there was no report on haemogregarine infection in *Rana cyanophlyctis* except the one, *Haemogregarina berestneffi* Castellani and Willey, communicated by the authors in the present monograph.

The blood parasite under consideration is noncapsulated. *H. berestneffi* though a parasite of the same host *R. cyanophlyctis*, cannot be compared with the haemogregarine described from Andaman Islands as the latter lacks a capsule. It can be categorised with those haemogregarines like *Haemogregarina* sp. Ball, 1967 from *R. wittei*, *Haemogregarina* sp. Kudo, 1922 from *R. pipiens* and *R. clamitans*, and *H. nucleobisecans* Shortt, 1917 from *Bufo melanostictus* because all of them exert similar impact on their host cells leading to their marked hypertrophy and cleaving of the host cell nucleus into two longitudinal halves. As *H. nucleobisecans* possesses a well defined capsule it can be safely kept away from the present parasite which is devoid of such a distinct feature. The other two unnamed haemogregarines with cleaved nucleus can be proximated with the parasite presented by the authors in this communication but a perusal of this morphometric measurements, cytoplasmic characters and hosts with their geographical segregation tempt the authors to isolate their described parasite from the aforesaid species and designate it as new species. So the name *Haemogregarina andamanensis* sp. nov. has been proposed for the new haemogregarine after its type locality.

A comparative review of *H. andamanensis* sp. nov. with other related members :

Parasite and reference	Capsule	Gametocyte		Nucleus		Host
		Length in μm	Breadth in μm	Length in μm	Breadth in μm	
<i>H. andamanensis</i> sp. nov.	Absent	14.55	3.45	2.05	1.7	<i>Rana cyanop-</i> <i>hlyctis</i>
<i>H. nucleobisecans</i> Shortt, 1917	Present	18.3	4.8	—	—	<i>Bufo melanos-</i> <i>tictus</i>
<i>Haemogregarina</i> sp. Ball, 1967	Absent	17.5	5.8	—	—	<i>R. wittei</i>
<i>Haemogregarina</i> sp. Kudo, 1922	Present	23.8	3.6	—	—	<i>R. pipiens</i>
	Present	27.6	2.4	—	—	<i>R. clamitans</i>

9. *Haemogregarina pulchra* sp. nov.

(Figs. 9a—e ; Plate V Fig. 12)

Type-host : *Kaloula pulchra* Gray

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

Type-material : On slides.

Holotype : Z. S. I., Reg. No. Pt. 2003

Paratypes : On the same slides.

Coll. Dr. R. Ray.

Description : The present description is based on the parasite obtained from *Kaloula pulchra*, collected from Purulia district, West Bengal, India. The different stages found in the blood are as follows :

Gametocytes : 1) Elongate mature form (Figs. 9a, b & Plate V Fig. 12, Table 13) : N=15. All the erythrocytic forms are considered as gametocytes. Each gametocyte is elongate and completely occupies one half of the R.B.C. ; concave side of the parasite proximates the convex side of the host cell nucleus or sometimes keep apart from it. It measures $15.41 \mu\text{m}$ (2.45) by $3.0 \mu\text{m}$ (0.76) with an area of $32.53 \mu\text{m}^2$ (7.76) ; occupying 33.4% of the total host cell-parasite complex. Anterior end is broad and round, sometimes a neck-like constriction may occur (Fig. 9a). The posterior end is attenuated and formed a hook-like structure encircling the host cell-nucleus, or sometimes rounded.

Cytoplasm hyaline, homogeneous, more granulated towards the broader anterior end ; stained light blue with Leishman and Giemsa. The anterior

end having some pink stained volutin granules which do not form any cap-like structure.

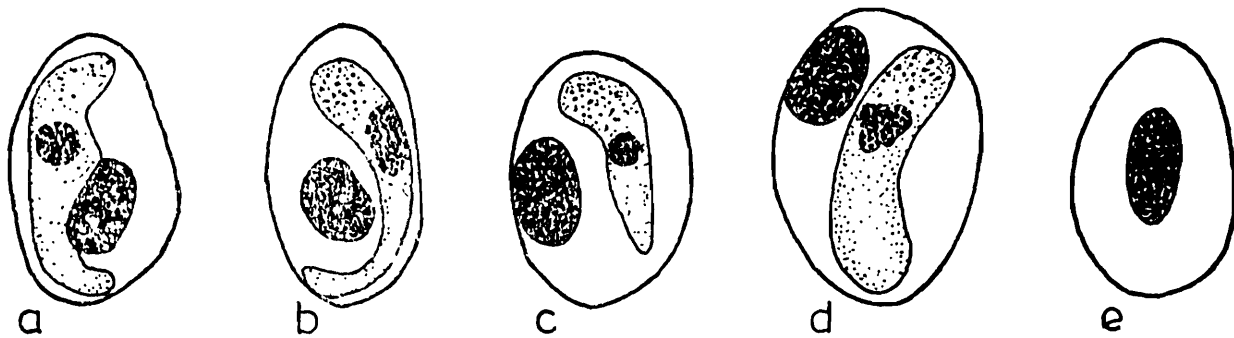


FIG. 9. HAEMOGREGARINA PULCHRA $\overline{10 \mu m}$
SP. NOV.

Fig. 9. Camera lucida drawings of the erythrocytic stages of *Haemogregarina pulchra* sp. nov. from *Kaloula pulchra*.

a—d, the elongate mature gametocytes ; e, the uninfected erythrocyte.

The nucleus is rounded or oval, sometimes elongated, measuring $2.3 \mu m$ (0.77) by $1.75 \mu m$ (0.36) with an area of $15.5 \mu m^2$ (1.0) ; central or subcentral in position ; stained red with Giemsa. Chromatin granules are compactly arranged. No karyosome is noted. The parasites are not enclosed in sheath or capsule.

2) Intermediate and broad forms (Fig. 9c) : N=15. Broad forms having both the ends rounded, measuring $15.5 \mu m$ by $4.0 \mu m$ with an area of $44.0 \mu m^2$. Cytoplasm stains deep blue with Leishman. Pink colour volutin granules were observed at the anterior end. Nucleus is subcentral towards the anterior end and round in structure. It measures $2.5 \mu m$ by $2.0 \mu m$ with an area of $5.7 \mu m^2$; chromatin granules are compactly arranged. Intermediate forms measure $10.5 \mu m$ by $2.5 \mu m$ with an area of $22.5 \mu m^2$, anterior end is rounded and granulated, posterior end is attenuated. The nucleus is rounded to oval measuring $2.0 \mu m$ by $1.5 \mu m$ with an area of $2.5 \mu m^2$; more or less central in position.

Extracorpuseular free gametocytes in the plasma could not be detected.

Effect of the parasite on the host cell : The host cells are much hypertrophied due to the infection of the parasite. The parasites fill the whole length of the R.B.C. thereby pushing the host cell nucleus aside. Hypertrophy with great displacement of the nucleus is another important feature of the parasites. The host cell nucleus is either pushed by the side of the R.B.C. or sometimes it may be found at the poles which is of unusual

occurrence. Double or multiple infection was not recorded. N.D.R.=0.24 (0.10). Host cell hypertrophied in length (18.3%) in width (15.2%) and in area (24.9%). The cell nucleus hypertrophied in area (44.1%).

Uninfected erythrocytes : N=15. Cell 13.1 μm (0.76) by 7.95 μm (0.85) and 77.85 μm^2 (5.76) in area. Cell nucleus 5.9 μm (0.60) by 2.52 μm (0.36) and 10.67 μm^2 (1.38) in area. N.D.R.=0.98 (0.04).

Schizogony and vector unknown.

Prevalence : Out of 6 examples of *Kaloula pulchra*, collected from Santaldi, Purulia district, West Bengal, India, 2 were found to be infected with the present parasite in their blood. A minimum of 1% to a maximum of 2.5% of the total R.B.C. counted were found positive for the parasites.

Remarks : A perusal of literature revealed that there was no report of *Haemogregarina* from *Kaloula pulchra* which belongs to the family Microhylidae except the one *H. kaloulae* sp. nov. which has been described from a subspecies *Kaloula pulchra taprobanica* Parker by the present authors.

The parasite under report differs from *H. kaloulae* sp. nov. by not having any sheath or capsule and polar "cap", and being smaller in size. Instead, the parasite from *K. pulchra* contains some volutin granules on the broader anterior end.

Considering the strong familial specificity and characteristic morphologies represented by the present parasite, a new name *Haemogregarina pulchra* sp. nov. has been coined after the specific name of its host.

10. ***Haemogregarina systoma*** sp. nov.
(Fig. 10 a—e ; Plate V Figs. 13, 14)

Type-host : *Uperodon systoma* Schneider.

Type-locality : Purulia district, West Bengal, India.

Type-material : On slides.

Holotype : Z. S. I. Reg. No. Pt. 2004

Paratypes : On the same slide.

Coll. Dr. R. Ray.

Description : The parasitic protozoa described hereunder was obtained from an Indian burrowing frog *Uperodon systoma*, collected from the Purulia district, West Bengal, India.

Gametocytes : Elongate mature form (Fig. 10a—d ; Plate V Figs. 13, 14, Table 14) : N=15. The parasite is elongated with both the ends rounded, in some cases one end may be attenuated and forming hook-like structure. The elongate form measures $15.3 \mu\text{m}$ (1.12) by $3.9 \mu\text{m}$ (0.15) with an average area of $50.7 \mu\text{m}^2$ (5.60) ; occupying 44.2% of the total host cell parasite complex. The parasites occupy almost complete one half of the R.B.C. ; the concave side of the parasite may face the convex side of the host cell nucleus or opposite of it ; seldom the host cell nucleus is displaced at one corner.

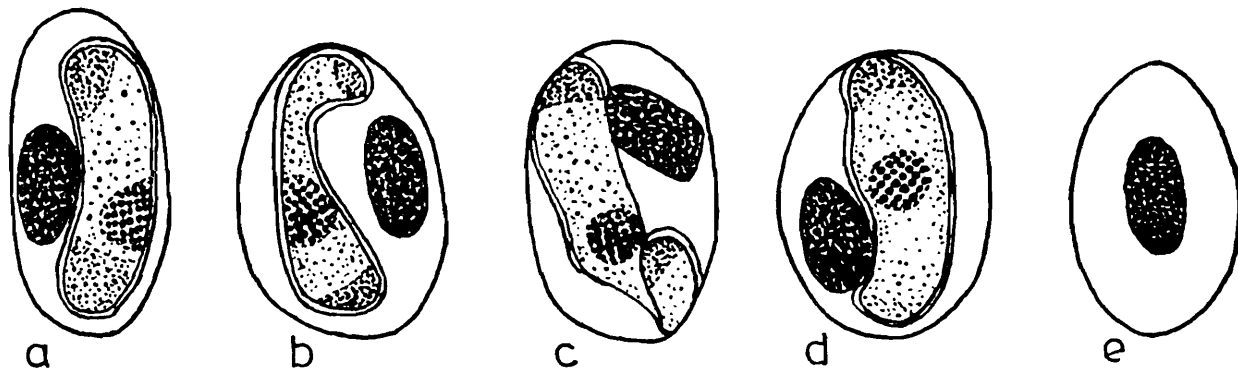


FIG. 10. HAEMOGREGARINA SYSTOMA $\overline{10 \mu\text{m}}$
SP. NOV.

Fig. 10. Camera lucida drawings of the erythrocytic stages of *Haemogregarina systoma* sp. nov. from *Uperodon systoma*.

a—d, the elongate capsulated mature gametocytes ; e, the uninfected erythrocyte.

The cytoplasm stained deep blue with Wright and Giemsa stains. It is homogeneous and coarsely granular. The volutin granules are found to be distributed throughout the body of the parasite. There are polar "caps" [measuring $7.3 \mu\text{m}^2$ (0.54) in area] on both the ends or sometimes at one end only (Figs. 10 a ; b ; Plate V Figs. 13, 14).

The nucleus is round, oval or triangular, measuring $3.4 \mu\text{m}$ (0.55) in length and $2.4 \mu\text{m}$ (0.40) in width with a mean area of $6.2 \mu\text{m}^2$ (0.95), situated at the centre ; stained deep red or purple colour with Wright and Leishman. It contains a large number of chromatin granules with definite pattern so as to form reticular structure and this is the special characteristic feature of the nucleus in the present parasite. Karyosome has not been detected.

The parasite is always enclosed by a deep red stained sheath which measures $15.7 \mu\text{m}$ by $4.2 \mu\text{m}$ and $55.5 \mu\text{m}^2$ in area.

No intermediate or young and free stages have been found in the blood. Presence of large number of elongated mature gametocytes denote the chronic phase of the infection.

Effect of the parasite on the host cell: Host cells are very much hypertrophied due to the infection of large elongate forms. The host cell nucleus is always displaced to the side of the erythrocytes, but sometimes towards one end. Multiple infection of the host cell was not observed. N.D.R=0.17 (0.09). Host cell hypertrophied in length (20.4%) in width (8.7%) and in area (13.7%). The host cell-nucleus hypertrophied in area (17.6%).

Movement in fresh preparation: When a fresh sample of blood mixed with saline citrate solution was examined, some parasites were found to loop or uncoil within the erythrocytes. They were never seen to come out of the host cells.

Uninfected erythrocytes: N=15. Cell 14.7 μm (0.97) by 9.2 μm (0.50) and 99.8 μm^2 (5.32) in area. Cell nucleus 6.1 μm (0.47) by 2.8 μm (0.88) and 13.6 μm^2 (1.79) in area. N.D.R=0.96 (0.05).

Schizogony and vector unknown.

Prevalence: Out of 17 examples of *Uperodon systoma* collected from Purulia district, West Bengal, 4 were found to be infected with the parasite. A minimum of 1% to a maximum of 3% of the total R.B.C. counted were found to be positive for the parasite.

Remarks: The present record of *Haemogregarina* is for the first time from the anuran host genus *Uperodon* and third from the family Microhylidae. The other two species *H. kaloulae* and *H. pulchra* of the same family have been included in this present monograph.

The parasite under report has got certain resemblances with *H. aegyptia* Mohammed and Mansour, 1963, *H. nucleobisecans* Shortt, 1917 and *H. pestanae* Franca, 1911 in possessing similar polar cap-like structure but differs from them in the following morphometric parameters.

This parasite is also comparable with *H. magna* in having definite capsule and *H. berestneffi* Castellani and Willey, 1905 by the nuclear structure and polar caps.

Due to differences in the gametocytes morphology amongst the species of the genus *Haemogregarina* and viewing the specificity of this parasite

at the family level, the present parasite under communication from the amphibia *Uperodon systoma*, an Indian burrowing frog, appears to be distinct. Therefore a new name *Haemogregarina systoma* sp. nov. has been proposed after its host's name to mark the specific identity.

Parasite and reference	Gametocyte		Nucleus		Host
	Length	Breadth	Length	Breadth	
	in μm	in μm	in μm	in μm	
<i>Haemogregarina systoma</i> sp. nov.	15.3	3.9	3.4	2.4	<i>Uperodon systoma</i>
<i>H. aegyptia</i> Mohammed and Mansour, 1963	14	7.3	3.7	2.9	<i>Bufo regularis</i>
<i>H. nucleobisecans</i> Shortt, 1917	18.3	4.8	—	—	<i>B. melanostictus</i>
<i>H. pestanae</i> Franca, 1911	12	4.5	3.7	3.7	<i>B. regularis</i>

11. *Haemogregarina* sp. Sinha

1979. Sinha, C. K. *Proc. 2nd Nat. Cong. Parasitol.*, B. H. U., Varanasi, p. 58.

Host : *Bufo himalayanus* Gunther.

Locality : Darjeeling (altitude 200 m.), West Bengal, India.

Prevalence : Out of 25 Himalayan toads, *Bufo himalayanus* examined, 2 were found to be infected with the parasite.

Description : These are non pigmented intra-erythrocytic haematozoa. The blood forms were mainly gametocytes.

Remarks : Sinha (1979) reported the occurrence of a *Haemogregarina* in the Himalayan toads *Bufo himalayanus* for the first time but he neither named nor described the parasite with detail morphometric measurements. While surveying the protozoan parasites of North Bengal the senior author collected a number of Himalayan toads from Darjeeling and its surroundings but no haemogregarine could be detected.

TAXONOMIC PROBLEMS OF ANURAN HAEMOGREGARINES

It is one of the most difficult task for the protozoologists to establish the correct identity of the haemogregarines at the species level and even at the genus level because of the paucity of an authentic classification of these blood parasites of the poikilotherms. Generic and specific identity are determined based on the details of the life-cycle which are, in most cases, very incompletely known.

Members of the genus *Haemogregarina* are blood coccidians belonging to order Eucoccida, suborder Adelaina (Honigberg *et. al.* 1964), and possess

the features of these taxonomic categories. The developmental stages of haemogregarines parasitizing the erythrocytes of cold-blooded vertebrates are merozoites, schizonts, trophozoites and gametocytes like plasmodium. These forms usually stain well with Romanowsky-type stains, the cytoplasm responds to shades of blue and the nucleus to shades of red, which are in definite contrast to the red blood cells.

The characteristic features used as systematic criteria for classifying the genus *Haemogregarina* are briefly outlined below.

a) *Gametocytes* : The morphology of the gametocytes, unfortunately, does not provide a reliable clue for generic differentiation. Their morphological characteristics, together with other relevant data i.e. morphometric measurements, area measurements etc. provide reliable basis for specific identification. In some species the gametocytes show diagnostic features in their staining reactions, differentiation of macro-and micro-gametocytes and presence of polar cap and capsule etc. For example, the very slender form of *Haemogregarina gracilis* Wenyon, 1909, the huge body of *H. hamata* Garnham, 1950, the hooked anterior end of *H. boueti* Franca 1911, are all distinctive characters. The parasite nucleus also shows characteristic configuration in different species. These differences in morphology of gametocytes of various species may reflect, pronounced differences in the unknown part of the developmental cycle of the parasites.

b) *Effect of Gametocyte on Host cell* : Parasitized blood corpuscles often undergo considerable change in their shape, size and structure. Linear hypertrophy of the erythrocytes is enormous in large gametocytes of *H. magna*, while in this species as well as in *H. berestneffi* and in many others, the infected red blood corpuscles assume characteristic forms. In one of the toad's haemogregarines studied by Mohammed and Mansour (1966a) the infected corpuscles became wrinkled and appeared as a collapsed, empty sac. The host cell nucleus is also susceptible to a variety of changes in their shape, size, and position in the infected corpuscle. The formula, Nuclear Displacement Ratio (NDR) and percentage of host cell hypertrophy adopted in this connection proved to be useful criteria for specific diagnosis. The effects produced by the parasite on the host cell and its nucleus are in many cases constant and may add to the diagnostic characteristics of the species.

There are certain species of haemogregarine (*H. boueti*, *H. andamanensis*) which either fragment or cleave the host cell nucleus.

c) *Schizogony* : This general term is usually used to designate merogonic cycle of the parasite either in the erythrocyte or in the tissue cells of vertebrate host, which ultimately leads to the production of merozoites.

A considerable number of haemogregarine species have been recorded from amphibians, and it is surprising that schizogonic development has been so rarely reported. Attempts to discover the schizogonic stages often fail, even after careful search for developmental stages. A possible explanation may be that active schizogony, when developmental stages are present in reasonably large numbers, occurs only during a restricted period of infection, that escapes detection. Furthermore, the material to be examined may prove exhaustive, since any type of tissue in the host body may be the preferred site for the multiplication of the particular parasite.

In *Haemogregarina berestneffi* and *H. magna* lung is the site of their schizogony whereas in *H. nucleobisecans* and *H. kaloulae* schizogony occurs in the liver. Mohammed and Mansour (1966a, b) found two types of schizogony in *H. boueti* and *H. pestanae* of *Bufo regularis* which occurred in the liver. Presence of two types of schizonts, producing macro- and micro-merozoites, is characteristic of many species of haemogregarines but is absent, or not detected, in others.

So the haemogregarines differ from each other in their merogony, in localization of schizonts in a particular part or parts of the host body, types of cells harbouring developmental forms and morphological details of schizonts and merozoites.

d) *Sporogony* : Knowledge concerning sporogony of haemogregarines is even poorer than that of schizogony. The problem involves the discovery of the vector (leech in amphibia) that may feed on the blood of the host, demonstrating the sporogonous development in this vector, and determining the natural mode of infection. Sporogony has been worked out for only a few of the known species of reptilian haemogregarines, the vast majority of which await further investigations on this part of their life-cycle. In amphibian haemogregarines no sporogony could be detected until now.

Although most of the diagnostic characters discussed above cannot be taken as decisive systematic criteria at a generic level, they are very useful for identifying the species (Mohammed and Mansour, 1959). In most cases only gametocytes are encountered and special care should be given to describing their morphology. The shape, cytoplasmic and nuclear structure, staining reactions and morphometric measurements of gametocytes

are reliable criteria for specific identification. Biological characteristics, particularly the effect of the gametocytes on their host cells and nuclei, may also be considered. Merogonic and sporogonic stages add to the characteristics of the species and complete its morphological picture.

Members of the genus *Haemogregarina* infecting one amphibian family are not normally infective to other families. This family specificity may be considered as an important guide to the identification of haemogregarines because there is no evidence to the contrary of the above contention available both under natural as well as under experimental condition.

It is clear from the review presented above that in the majority of cases few haemogregarine characteristics provide a reliable basis for definitely relating a certain parasite to the genus *Haemogregarina*. Details of the sporogonous cycle seem to be the only reliable criterion since they are the 'Key-stone' in the system of classification. Unfortunately, these cycles are usually difficult to determine.

So the genus *Haemogregarina* subsequently became a depository for many insufficiently known forms, all with the common features of parasitizing the blood cells of the vertebrates and all presumably being heteroxenous. This confusion originated because the development cycles and transmission of most "haemogregarines", particularly those of poikilothermous vertebrates, have never been elucidated.

Current diagnosis of the genus *Haemogregarina* (*sensu strictu*) specified the occurrence of schizogony in the cells of the circulating blood. But it cannot be properly followed in each case. In such cases the name *Haemogregarina* may mean little more than the general term "haemogregarine" and the generic name is used in its broad (*sensu latu*). This usage, with a proper indication (in Garnham, 1950) is a useful conservative procedure and more correct than relating a species of haemogregarine to the genus *Hepatozoon* or *Karyolysus* on provisional basis.

In the present investigation on the blood parasites of Indian anurans the authors propose to use the generic term *Haemogregarina* (*sensu strictu*) for those species which exhibit typical schizogony in the vertebrate and *Haemogregarina* (*sensu latu*) for the other haemogregarines in which case schizogony could not be observed.

Eleven species of Haemogregarines from the Indian anuran hosts have been communicated in the present monograph of which six have been

proposed to be new to science. Out of these eleven species, *Haemogregarina berestneffi*, *H. magna*, *H. perinucleophilum* and *H. kaloulae* sp. nov. are considered by the present authors to belong to *Haemogregarina* (*sensu strictu*) and the rest seven *H. nucleobisecans*, *H. maculatus* sp. nov., *H. pattoni* sp. nov., *H. andamanensis* sp. nov., *H. pulchra* sp. nov., *H. systoma* sp. nov., and one unnamed species have been included under *Haemogregarina* (*sensu lato*) in which erythrocytic schizogony has not been observed.

SUMMARY

1. In the present communication the authors described 11 species of *Haemogregarina* viz., *Haemogregarina berestneffi*, *H. magna*, *H. nucleobisecans*, *H. perinucleophilum*, *H. maculatus* sp. nov., *H. pattoni* sp. nov., *H. kaloulae* sp. nov., *H. andamanensis* sp. nov., *H. pulchra* sp. nov., *H. systoma* sp. nov., and an unnamed haemogregarine which have been recorded so far from India. Of these 6 have been christened with new species name.

2. *Haemogregarina berestneffi* is described from two new anuran hosts, *Rana cyanophlyctis* and *R. limnocharis* from a new geographical locale (West Bengal, India). Erythrocytic schizogony and tissue schizogony of this parasite have been communicated here for the first time.

3. *Haemogregarina magna* is also redescribed with detail morphometric measurements and schizogony both in the erythrocytes as well as in the endothelial cells of lung tissue of *Rana tigrina*.

4. *Haemogregarina nucleobisecans* with hepatic schizogony is described from a new geographical locale of Eastern India (Purulia) and from a new host *Bufo andersoni* along with *B. melanostictus*.

5. *Haemogregarina perinucleophilum* has also been described with detail morphometric measurements and erythrocytic schizogony from *Rana tigrina*.

6. *Haemogregarina maculatus* sp. nov. from *Rhacophorus maculatus*, *H. pattoni* sp. nov. from *Rana hexadactyla*, *H. kaloulae* sp. nov. from *Kaloula pulchra taprobanica*, *H. andamanensis* sp. nov. from *Rana cyanophlyctis*, *H. pulchra* sp. nov. from *Kaloula pulchra*, *H. systoma* sp. nov. from *Uperodon systoma* and an unnamed haemogregarine from *Bufo himalayanus* have been described with detail morphometric measurements.

7. The taxonomic problem of anuran haemogregarines has been discussed and a host-parasite list of the recorded species of Anuran Haemogregarines in India has also been added at the end of the monograph.

ACKNOWLEDGEMENT

The authors are very much thankful to Dr. B. K. Tikader, Director, Zoological Survey of India, for providing laboratory facilities to the senior author and for his interest in this work.

Thanks are also due to Drs. A. K. Mandal, A. K. Das, N. C. Nandi, A. Bhattacharya, C. B. Srivastava, Zoological Survey of India for various suggestions and constructive criticism rendered from time to time during this work.

Best thanks are due to Prof. Norman D. Levine, College of Veterinary Medicine and Parasitology, University of Illinois, Urbana, U.S.A., for his wise suggestions, valuable comments and helping with reprints, microcards etc. from time to time throughout the study.

The co-operation of Sarbasri F. N. Murmu and M. K. Sengupta (Artist) for their technical assistance in this work is thankfully acknowledged.

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TABLE 1. Comparative morphometric measurements of Gametocytes of *H. berestneffi* in *Rana limnocharis* and *Rana cyanophlyctis*.

	<i>Rana limnocharis</i>				<i>Rana cyanophlyctis</i>			
	M	SD	SE	CV	M	SD	SE	CV
Gametocyte								
Length (μm)	16.5	0.76	0.24	4.60	11.5	0.79	0.32	6.86
Width (μm)	2.57	0.32	0.10	12.45	3.06	0.79	0.32	6.86
Area (μm^2)	29.56	3.18	1.0	10.75	36.16	4.14	1.69	11.45
Nucleus								
Length (μm)	2.85	0.39	0.12	13.68	2.45	0.65	0.26	26.50
Width (μm)	2.19	9.22	0.06	10.04	1.96	0.29	0.12	14.79
Area (μm^2)	5.13	0.76	0.24	14.81	5.70	2.36	0.97	41.10
Capsule								
Length (μm)	18.3	1.60	0.50	8.74	12.5	0.61	0.25	4.88
Width (μm)	3.14	0.27	0.08	8.59	3.8	1.31	0.53	34.47
Area (μm^2)	31.5	4.06	2.10	10.50	36.95	5.04	2.06	13.64
Parasitized Erythrocyte								
Length (μm)	14.05	0.43	0.13	3.06	13.90	0.53	0.21	3.81
Width (μm)	10.50	0.83	0.26	7.90	7.83	0.37	0.15	4.72
Area (μm^2)	101.45	12.04	3.81	11.86	80.0	6.70	2.74	8.37
Nucleus								
Length (μm)	5.1	0.63	0.19	12.35	5.08	0.53	0.21	10.43
Width (μm)	3.61	0.48	0.15	13.29	3.08	0.67	0.27	21.75
Area (μm^2)	13.97	2.12	0.67	15.17	12.66	0.74	0.30	5.84
Unparasitized Erythrocyte								
Length (μm)	12.9	0.37	0.11	2.86	12.1	0.37	0.15	3.05
Width (μm)	9.1	0.53	0.16	5.82	8.2	0.50	0.20	6.09
Area (μm^2)	86.97	9.41	2.97	10.11	71.2	2.20	0.90	3.08
Nucleus								
Length (μm)	5.3	0.60	0.19	11.32	3.34	0.20	0.08	5.98
Width (μm)	3.14	0.27	0.08	8.59	3.8	1.31	0.53	34.47
Area (μm^2)	31.5	4.06	2.10	10.50	36.95	5.04	2.06	13.64

TABLE 2. Morphological measurements of the erythrocytic stages of *H. magna* in *Rana tigrina*, expressed as mean, standard deviation (SD), standard error (SE) and coefficient of variation (CV). Linear measurements in micrometres and area measurements in micrometres²

	Mean	SD	SE	CV
Uninfected RBC				
Length	15.6	1.26	0.40	8.07
Width	8.5	0.52	0.16	6.11
Area	94.4	12.95	4.10	13.71
Nuclear area	12.2	1.92	0.60	15.73
*NDR	0.92	0.06	0.02	6.52
Infected RBC				
Length	15.1	0.83	0.26	5.49
Width	9.3	0.41	0.13	4.40
Area	104.2	8.02	2.53	7.69
Nuclear area	18.3	2.81	0.89	15.35
*NDR	0.22	0.15	0.05	8.18
Intracorpuseular Parasite (Gameteocyte)				
Length	12.4	1.97	0.35	8.95
Width	4.06	0.23	0.07	5.66
Area	44.5	7.09	2.24	15.93
Nuclear area	12.1	1.93	0.61	15.95
Extracorpuseular parasite (Free Gameteocyte)				
Length	11.4	1.68	0.56	14.73
Width	4.16	0.23	0.07	5.52
Area	47.7	6.81	2.27	14.27
Nuclear area	12.21	2.05	0.68	16.78
Erythrocytic schizont				
Length	12.5	1.21	0.45	9.68
Width	10.7	1.43	0.54	13.36
Area	103.9	7.03	2.51	8.69
Merozoite				
Length	1.90	0.16	0.05	8.42
Width	1.05	0.15	0.04	14.28
Area	1.30	0.21	0.06	16.15

*NDR=Nuclear Displacement Ratio.

TABLE 3. Comparative measurements of gametocytes of *H. magna* from *R. pipiens* (reproduced from Levine and Nye, 1977) and *R. tigrina* from the present study.

	<i>Rana pipiens</i>		<i>Rana tigrina</i>	
	Mean	SD	Mean	SD
Parasite (Gametocyte)				
Length (μm)	18	—	12.4	1.97
Width (μm)	6	—	4.06	0.23
Area (μm^2)	—	—	44.5	7.09
Nucleus				
Length (μm)	4	—	4.4	0.67
Width (μm)	4	—	3.3	0.28
Area (μm^2)	—	—	12.1	1.93
Parasitized Cell (erythrocyte)				
Length (μm)	24	—	15.1	0.83
Width (μm)	14	—	9.3	0.41
Area (μm^2)	—	—	104.2	8.02
Host cell nucleus				
Length (μm)	13	—	6.7	0.64
Width (μm)	4	—	3.2	0.42
Area (μm^2)	—	—	18.3	2.81
*NDR	—	—	0.22	0.15

*Nuclear Displacement Ratio. SD=Standard deviations.

TABLE 4. Comparative measurements of gametocytes of *Haemogregarina nucleobisecans* from *Bufo melanostictus* and *Bufo andersoni*. Linear measurements in micrometers.

	<i>B. melanostictus</i>				<i>B. andersoni</i>			
	Mean	SD	SE	CV	Mean	SD	SE	CV
Mature gametocyte								
Length	14.4	1.71	0.54	11.87	13.8	1.30	0.41	9.42
Width	2.95	0.26	0.08	8.81	2.50	0.44	0.14	17.60
Area	28.95	3.77	1.19	13.03	24.97	4.52	1.43	18.10
Nucleus								
Length	3.25	0.81	0.25	24.92	2.67	0.52	0.16	91.47
Width	1.95	0.41	0.13	21.02	1.90	0.20	0.06	10.52
*Area	5.18	1.46	0.46	28.18	4.37	0.73	0.23	16.70
Capsule								
Length	15.17	1.82	0.57	11.99	14.72	1.12	0.35	7.60
Width	3.7	0.40	0.12	10.81	3.10	0.48	0.15	15.48
*Area	31.84	4.28	1.35	13.44	27.59	5.55	1.75	20.11
Infected erythrocyte								
Length	16.60	1.01	0.32	6.08	14.55	0.35	0.11	2.40
Width	8.52	0.52	0.16	6.10	9.08	0.77	0.24	8.49
*Area	111.47	7.38	2.33	6.62	97.24	9.21	2.91	9.47
Nuclues								
Length	6.16	0.29	0.09	4.70	5.50	0.31	0.10	5.63
Width	3.33	0.36	0.11	10.91	3.05	0.26	0.08	8.52
*Area	15.54	1.50	0.47	9.65	13.21	1.84	0.58	13.92
**NDR	0.17	0.15	0.01	88.0	0.24	0.13	0.04	54.16
Uninfected erythrocyte								
Length	15.47	0.79	0.25	5.10	12.35	0.22	0.07	1.78
Width	8.55	0.35	0.21	4.09	9.05	0.26	0.08	2.87
*Area	100.10	10.69	3.38	10.67	91.28	6.45	2.04	7.06
Nucleus								
Length	5.85	0.22	0.07	3.76	4.84	0.28	0.09	5.78
Width	2.72	0.29	0.09	10.66	2.63	0.24	0.07	9.12
*Area	13.28	1.66	0.52	12.50	10.91	0.98	0.31	8.98
**NDR	0.97	0.04	0.01	4.12	1.0	—	—	—

*=Area in μm^2 **=Nuclear Displacement Ratio. SD=Standard Deviation
SE=Standard Error. CV=Coefficient of variation.

TABLE 5. Comparative morphometric measurements of eight related species of *Haemogregarina* from the family Bufonidae. (All measurements in micrometers).

	Parasite		Nucleus		Infected R.B.C.		Normal R.B.C.	
	Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth
1. <i>H. nucleobisecans</i>								
Shortt, 1917								
Range	—	—	—	—	—	—	—	—
Average	18.3	4.8	—	—	—	—	—	—
<i>H. nucleobisecans</i> (from the present study)								
Range	10.5-18	2.5-3.5	2-4.5	1.5-3	15-18	8-9.2	14-16.5	8-9
Average	14.4	2.95	3.25	1.95	16.6	8.52	15.47	8.55
2. <i>H. tunisiensis</i> Nicolle, 1904								
Range	12-15	—	—	—	—	—	—	—
Average	—	8	—	—	—	—	—	—
3. <i>H. aegyptia</i> Mohammed & Mansour, 1963								
Range	20.1-22.5	4.2-5.1	3.3-4.5	3.6-4.2	—	—	—	—
Average	21.3	4.65	3.9 in diam.	—	23.5	11.7	15.7	12.0
4. <i>H. boueti</i> Franca, 1911								
Range	19-26	5-6	4-6	3-6	20-24	8-15	12-19	10.13
Average	22.3	6.0	5.2	4.2	21	10.6	15.7	12.0
5. <i>H. pestanae</i> Franca, 1911								
Range	—	—	—	—	—	—	—	—
Average	12.0	4.5	3.7	3.7	—	—	—	—
6. <i>H. faiyensis</i> Mansour & Mohammed, 1966								
Range	13-17	4-5	3-5	3-5	—	—	—	—
Average	15.5	4.5	4.5	3.9	18.3	11.3	15.7	12.0
7. <i>H. moloensis</i> Hoare Franca, 1920								
Range	—	—	—	—	—	—	—	—
Average	25	3	—	—	—	—	—	—
8. <i>H. froilanoi</i> Franca, 1925								
Range	16.5-21	—	—	—	—	—	—	—
Average	—	4.5	6.7	5.0	—	—	—	—

TABLE 6. Morphometric measurements of *Haemogregarina perinucleophilum* in *Rana tigrina*, expressed as Mean, Standard Deviation (SD), Standard Error (SE) and Coefficient of Variation (CV). Linear measurements micrometers and area measurements in micrometers²

	Mean	SD	SE	CV
Uninfected RBC				
Length	13	1.5	0.47	11.53
Width	8.6	0.82	0.26	9.53
Area	87.0	11.25	3.56	12.93
Nuclear area	11.10	2.25	0.71	20.27
*NDR	0.90	0.07	0.02	7.77
Infected RBC				
Length	12.8	2.18	0.69	17.03
Width	9.7	1.05	0.33	10.82
Area	87.7	16.42	5.19	18.72
Nuclear area	13.2	2.84	0.89	21.51
*NDR	0.26	0.21	0.06	80.76
Intracorporeal parasite (Gametocyte)				
Length	11.1	3.3	1.04	29.72
Width	3.1	0.76	0.24	24.50
Area	25.7	7.06	2.23	27.40
Nuclear area	6.0	1.90	0.60	31.60
Extracorporeal parasite (Gametocyte)				
Length	9.6	0.37	0.16	3.85
Width	2.6	0.37	0.16	3.85
Area	13.9	0.95	0.42	6.83
Nuclear area	2.0	0.26	0.11	13.0

*NDR = Nuclear Displacement Ratio.

TABLE 7. Comparative morphological measurements of the gametocytes of *H. berestneffi*, *H. magna* and *H. perinucleophilum* recorded from *Rana limnocharis* and *R. tigrina* in the present study. Linear measurements in micrometers and area measurements in micrometer² standard deviation in parenthesis.

	<i>H. berestneffi</i>	<i>H. magna</i>	<i>H. perinucleophilum</i>
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)
Intracorpuseular parasite (Gametocyte)			
Length	16.5 (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)	44.5 (7.09)	25.7 (7.06)
Nuclear area	5.13 (0.76)	12.1 (1.93)	6.0 (1.90)
Extracorpuseular parasite (Free Gametocyte)			
Length	17.6 (0.74)	11.4 (1.68)	9.6 (0.37)
Width	2.3 (0.22)	4.16 (0.23)	2.6 (0.37)
Area	27.7 (1.28)	47.7 (6.81)	13.9 (0.95)
Nuclear area	4.77 (1.34)	12.21 (2.05)	2.0 (0.26)

*NDR=Nuclear Displacement Ratio.

TABLE 8. Morphometric parameters of *Haemogregarina maculatus* sp. nov. from *Rhacophorus maculatus*, an Indian tree frog. Measurements indicate Mean (M), Standard deviation (SD), Standard error (SE) and Coefficient of variation (CV) of 15 gametocytes and erythrocytes.

	<i>Erythrocytes</i>			<i>Erythrocyte Nucleus</i>			<i>Parasite</i>			NDR
	Length μm	Width μm	Area μm^2	Length μm	Width μm	Area μm^2	Length μm	Width μm	Area μm^2	
Non-parasitized										
M	14.75	10.0	102.1	6.16	3.66	17.06	—	—	—	0.96
SD	1.10	0.20	5.56	0.37	0.47	2.03	—	—	—	0.03
SE	0.45	0.08	2.28	0.15	0.19	0.83	—	—	—	0.01
CV	7.45	2.0	5.44	6.0	12.84	11.83	—	—	—	3.12
Parasitized										
M	16.1	9.9	105.3	6.10	3.5	16.5	16.5	3.3	40.5	0.23
SD	1.14	0.79	8.02	0.27	0.52	1.18	0.68	0.61	6.09	0.15
SE	0.36	0.25	2.53	0.08	0.16	0.37	0.21	0.19	1.92	0.04
CV	7.08	7.97	7.61	4.42	14.85	7.15	4.12	18.48	15.03	65.21

Parasite nucleus $2.4 \mu\text{m}$ by $2.1 \mu\text{m}$ and $4.4 \mu\text{m}^2$ in area.

TABLE 9. A comparative morphometric measurements of the gametocytes of *Haemogregarina maculatus* sp. nov., *H. theileri* and *Haemogregarina* sp. (All measurements in micrometers).

	<i>H. maculatus</i> sp. nov. (<i>Rhacophorus maculatus</i>)		<i>H. theileri</i> Laveran, 1905 (<i>R. angolensis</i>)		<i>Haemogregarina</i> sp. Ball, 1967 (<i>R. wittei</i>)	
	Range	Mean	Range	Mean	Range	Mean
Intracorpuseular						
Gametocyte						
Length	15.5-17	16.5	—	18.9	—	17.5
Breadth	2-4	3.3	—	6.9	—	5.8
Area (μm^2)	29-52.7	40.5	—	—	—	—
Parasite Nucleus						
Length	2-3	2.4	—	—	—	—
Breadth	2-2.5	2.1	—	—	—	—
Area (μm^2)	3.5-5.7	4.4	—	—	—	—
Extracorpuseular						
Gametocyte						
Length	14-17	15.2	—	19.3	—	20.4
Width	2.5-3	2.95	—	2.4	—	3.5
Area	28-35	29.73	—	—	—	—
Nuclear are (μm^2)	5-6.5	5.57	—	—	—	—
Capsule	Absent		Present		Absent	
Beak or nipple-like structure	Present in intracorpuseular gametocyte		Present at one pole		Absent	

TABLE 10. Morphometric measurements of *Haemogregarina pattoni* sp. nov. in *Rana hexadactyla*, expressed as Means (M), Standard Deviation (SD), Standard Error (SE) and Coefficient of Variation (CV). Linear measurements in micrometers and area measurements in micrometer²

	M	SD	SE	CV
Uninfected RBC				
Length	11.1	0.14	0.04	1.26
Width	7.7	0.78	0.24	10.12
Area	62.8	3.08	0.97	4.90
Nuclear area	9.7	1.36	0.43	14.02
*NDR	0.96	0.06	0.02	6.25
Infected RBC				
Length	13.05	0.98	0.31	7.50
Width	8.70	1.05	0.33	12.06
Area	90.6	7.54	2.38	8.32
Nuclear area	10.9	1.91	0.60	17.52
*NDR	0.21	0.11	0.04	52.38
Intra corpuscular Parasite (Gametocyte)				
Length	9.70	2.76	0.87	28.45
Width	4.93	1.20	0.40	26.16
Area	33.05	8.30	2.76	25.11
Nucleus				
Length	3.42	1.01	0.33	29.53
Width	2.50	1.30	0.43	52.0
Area	5.90	2.63	0.87	44.57
"Cap" or band				
Length	6.0	2.02	0.71	33.6
Width	1.25	0.35	0.12	28.0
Area	5.15	2.25	0.80	43.68

TABLE 11. Morphometric measurements of *Haemogregarina kaloulae* sp. nov. in *Kaloula pulchra taprobanica*, expressed as Means (M); Standard Deviation (SD), Standard Error (SE) and Coefficient of Variation (CV). Linear measurements in micrometers and area measurements in micrometer²

	M	SD	SE	CV
Uninfected RBC				
Length	15.65	0.80	0.25	5.11
Width	8.92	0.49	0.15	5.49
Area	95.82	6.08	1.92	6.34
Nuclear area	12.2	1.77	0.56	14.50
*NDR	0.96	0.05	0.01	5.20
Infected RBC				
Length	17.92	1.28	0.40	7.14
Width	8.82	0.64	0.20	7.25
Area	113.09	6.79	2.15	6.00
Nuclear area	14.73	2.66	0.84	18.05
*NDR	0.14	0.10	0.03	71.42
Intracorpousclar Parasite (Gametocyte)				
Length	16.22	1.22	0.28	7.61
Width	3.65	0.51	0.16	13.97
Area	43.22	6.66	2.11	15.40
Nuclear area	6.12	1.68	0.53	27.45
Extracorpouscular Parasite (Free gametocyte)				
Length	17.03	0.28	0.08	1.64
Width	3.16	0.22	0.07	6.96
Area	45.69	2.29	0.72	5.01
Nuclear area	5.13	0.62	0.19	12.08
Parasites polar Cap				
Length	3.75	0.43	0.15	11.46
Width	2.87	0.59	0.21	20.55
Area	8.75	1.92	0.68	21.94

*Nuclear displacement ratio.

TABLE 12. Morphometric measurements of *Haemogregarina andamanensis* sp. nov. in *Rana cyanophlyctis*, expressed as Mean (M), Standard deviation (SD), Standard error (SE), and Coefficient of variation (CV). Linear measurements in micrometers and area measurements in micrometer²

	M	SD	SE	CV
Uninfected RBC				
Length	15.22	0.84	0.26	5.51
Width	9.97	0.39	0.12	3.91
Area	109.63	9.82	3.10	8.95
Nuclear area	12.82	3.82	1.20	29.97
*NDR	1.01	0.10	0.03	9.90
Infected RBC				
Length	15.95	1.33	0.42	8.33
Width	9.35	1.55	0.49	16.57
Area	125.41	14.55	4.60	15.24
Nuclear area	17.07	3.94	1.24	23.08
*NDR	0.4	0.27	0.09	67.50
Intracorpuseular parasite (Gametocyte)				
Length	14.55	1.76	0.55	12.09
Width	3.45	0.56	0.17	16.23
Area	45.69	5.09	1.61	11.14
Nuclear area	2.85	0.80	0.25	28.07
Extracorpuseular parasite (Free gametocyte)				
Length	15.60	0.36	0.15	2.30
Width	4.25	0.25	0.10	5.88
Area	54.76	1.29	0.53	2.35
Nuclear area	2.41	0.44	0.18	18.25

*NDR=Nuclear Displacement Ratio.

TABLE 13. Morphometric measurements of *Haemogregarina pulchra* sp. nov. in *Kaloula pulchra*, expressed as Means (M), Standard Deviation (SD), Standard Error (SE) and Coefficient of Variation (CV). Linear measurements in micrometers and area measurements in micrometer²

	M	SD	SE	CV
Uninfected R.B.C.				
Length	13.1	0.76	0.24	5.80
Width	7.95	0.85	0.26	10.69
Area	77.85	5.76	1.82	7.39
Nuclear area	10.67	1.38	0.43	12.93
*NDR	0.98	0.04	0.01	4.08
Infected R.B.C.				
Length	15.5	1.0	0.40	6.45
Width	9.16	1.02	0.42	11.13
Area	97.26	12.8	5.25	13.16
Nuclear area	15.38	1.80	0.74	11.70
*NDR	0.33	0.24	0.10	72.22
Intracorpuseular gametocyte				
Length	15.41	2.45	1.00	15.89
Width	3.0	0.76	0.31	25.33
Area	32.53	7.76	3.18	23.04
Nucleus				
Length	2.33	0.77	0.31	33.04
Width	1.75	0.36	0.14	20.57
Area	3.76	1.48	0.61	39.36

NDR=Nuclear Displacement Ratio.

TABLE 14. Morphometric measurements of *Haemogregarina systoma* sp. nov. in *Uperodon systoma*, expressed as Mean (M), Standard Deviation (SD), Standard Error (SE) and Coefficient of Variation (CV). Linear measurements in micrometers and area measurements in micrometer²

	M	SD	SE	CV
Uninfected R.B.C.				
Length	14.7	0.97	0.31	6.59
Width	9.2	0.50	0.16	5.43
Area	99.8	5.32	1.68	5.33
Nuclear area	13.6	1.79	0.56	13.16
*NDR	0.96	0.05	0.01	5.20
Infected R.B.C.				
Length	17.7	1.48	0.46	8.36
Width	10.0	1.0	0.31	10.0
Area	113.5	11.02	3.49	9.70
Nuclear area	16.0	1.75	0.55	10.93
*NDR	0.17	0.09	0.03	52.94
Intracorpuseular Parasite (Gametocyte)				
Length	15.3	1.12	0.35	7.32
Width	3.90	0.15	0.04	3.84
Area	50.7	5.60	1.77	11.04
Nuclear area	6.2	0.95	0.30	15.32
Polar Cap of the Parasite				
Area	7.3	0.54	0.17	7.39

* = Nuclear Displacement Ratio.

TABLE 15. Host-parasite list of recorded species of Anuran haemogregarines in India.

Sl. No.	Name of the host	Name of the parasite	References
1.	<i>Bufo andersoni</i>	<i>H. nucleobisecans</i>	Ray (1979, 1982, 1983)
2.	<i>Bufo himalayanus</i>	<i>Haemogregarina</i> sp.	Sinha (1979)
3.	<i>Bufo melanostictus</i>	<i>H. nucleobisecans</i>	Shortt (1917), Wenyon (1926), Bhatia (1938), Ray (1979, 1982, 1983)
4.	<i>Rana cyanophlyctis</i>	<i>H. berestneffi</i> <i>H. andamanensis</i> sp. nov.	Ray (1979) Ray (1979)
5.	<i>Rana hexadactyla</i>	<i>H. berestneffi</i> <i>H. pattoni</i> sp. nov.	Patton (1908), Dobell (1910) Bhatia (1938), Ray (1979) Ray (1979)
6.	<i>Rana limnocharis</i>	<i>H. berestneffi</i>	Scott (1926), Bhatia (1938), Ray (1977, 1979)
7.	<i>Rana tigrina</i>	<i>Haemogregarina magna</i> <i>H. perinucleophilum</i> <i>H. berestneffi</i>	Patton (1908), Wenyon (1926) Bhatia (1938), Ray (1979) Tiwari and Ray (1981) Ray (1983b) Berestneff (1903), Castellani and Willey (1905), Dobell (1910), Wenyon (1926), Bhatia (1938), Ray (1979), Tiwari and Ray (1981)
8.	<i>Rhacophorus maculatus</i>	<i>H. maculatus</i> sp. nov.	Ray (1979)
9.	<i>Kaloula pulchra</i>	<i>H. pulchra</i> sp. nov.	Ray (1979)
10.	<i>Kaloula pulchra taprobanica</i>	<i>H. kaloulae</i> sp. nov.	Ray (1979)
11.	<i>Uperodon systema</i>	<i>H. systema</i> sp. nov.	Ray (1979)

Uninfected hosts : *Bufo stomaticus*, *Rana tigrina crassa*, *R. malabarica*, *R. limnocharis syhadrensis*, *R. beddomii*, *R. erythraea*, *R. brevipalmata*, *Microhyla ornata*, *Rhacophorus malabaricus*.

ERRATA

	Printed	To read
In Contents under Systematic Description Species No. 6	<i>pattioni</i>	<i>pattoni</i>
Page 36 4th Para 1st line	Fig. 8j	Fig. 8i
„ 37 1st Para 10th line	Fig. 8i	Fig. 8h
„ 27 „ 5th line	break-like	beak-like

PLATES

PLATE I

Figs. 1-13. Photomicrographs of the erythrocytic and tissue stages of *Haemogregarina berestneffi* from *Rana limnocharis*.

- | | |
|---|--------|
| Fig. 1. Gametocytes in the peripheral blood | X 650 |
| Fig. 2. Young broad form of gametocyte | X 1550 |
| Fig. 3. Elongate mature gametocyte | X 1550 |
| Fig. 4. Macrogametocyte (ma) and microgametocyte (mi).
Arrow indicates the releasing merozoites from a ruptured erythrocyte. | X 2300 |
| Fig. 5. Typical macrogametocyte of <i>H. berestneffi</i> | X 1550 |
| Fig. 6. A growing larger form within a much hypertrophied erythrocyte | X 2000 |
| Fig. 7. An erythrocyte with double infection | X 1700 |
| Fig. 8-9. The extracorpuseular free gametocytes | X 1550 |
| Fig. 10. A rounded schizont in the endothelial cells of lung capillaries | X 2650 |
| Fig. 11. A mature schizont with developing merozoites | X 2660 |
| Fig. 12. A large schizont with central residual mass | X 1000 |
| Fig. 13. A large schizont with developing merozoites at the periphery. | X 1000 |

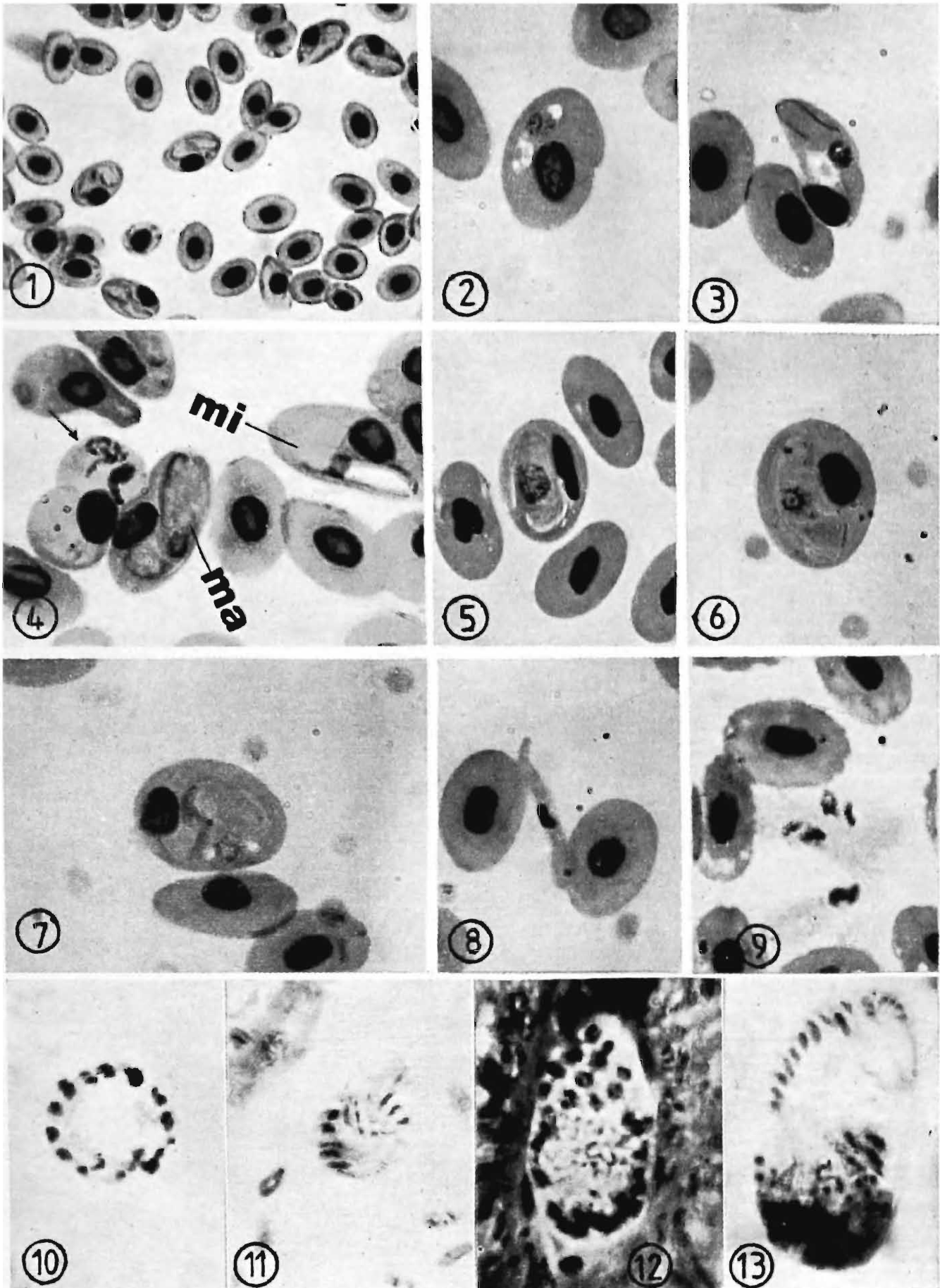


PLATE II

Figs. 1-13. Photomicrographs of erythrocytic and tissue stages of *Haemogregarina magna* from *Rana tigrina*.

Fig. 1. Gametocytes in the peripheral blood	X 600
Fig. 2 & 3. Macrogametocytes	X 1000
Fig. 4. Microgametocyte	X 1000
Fig. 5. A releasing macrogametocyte	X 1000
Fig. 6. An extracorpuseular free gametocyte	X 1250
Fig. 7. Erythrocytic schizont with 4 nuclei	X 2500
Fig. 8. Erythrocytic schizont with 10 nuclei	X 2504
Fig. 9. A mature erythrocytic schizont with 8 developing merozoites.	X 1750
Fig. 10. A mononucleated schizont in the section of lung tissue	X 1000
Fig. 11. A multinucleated schizont in the lung tissue	X 1006
Fig. 12. A mature schizont in the lung capillary	X 1000
Fig. 13. Arrow indicates the released merozoites in the lung tissue	X 1000

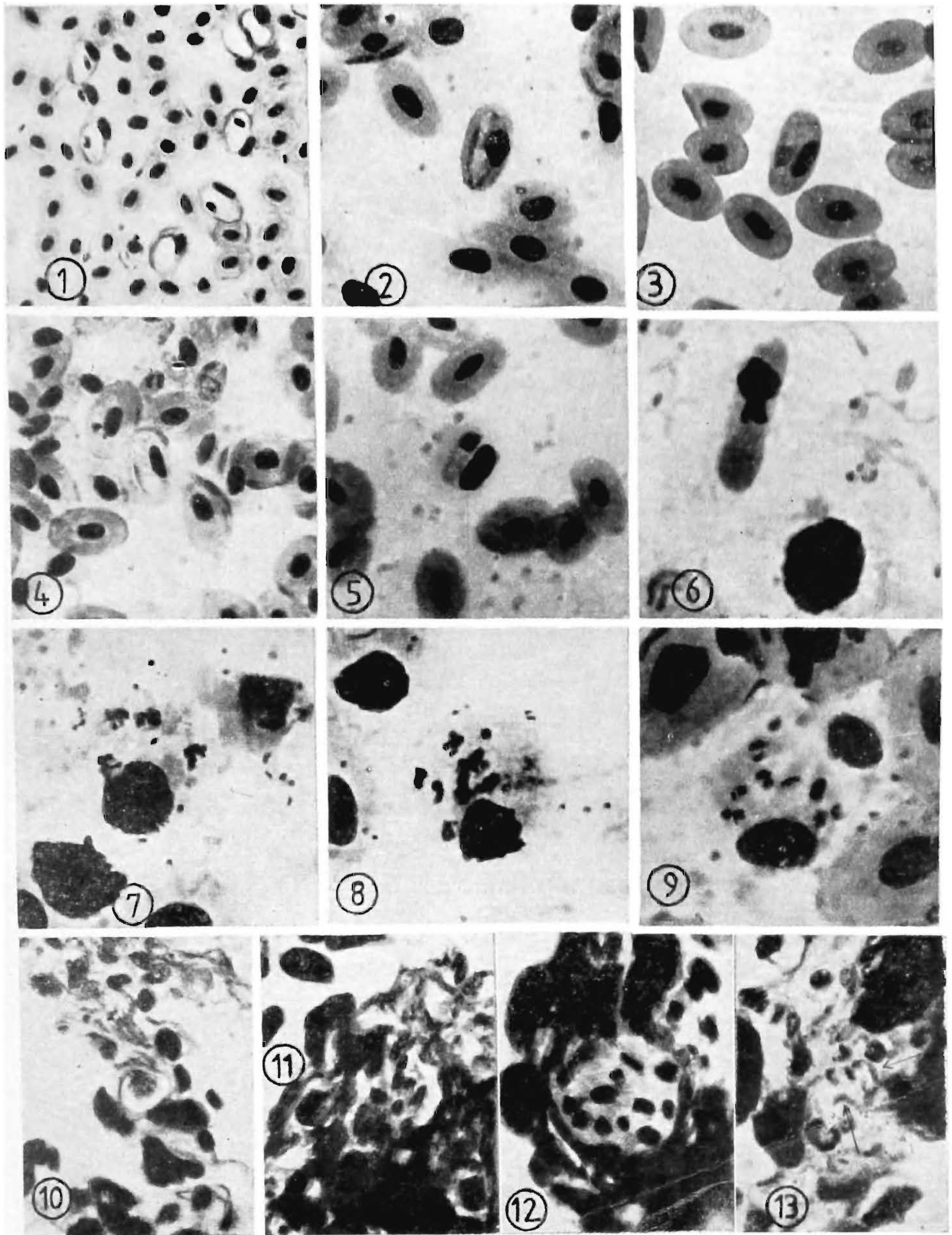


PLATE III

- Fig. 1-10. Photomicrographs of the erythrocytic and tissue stages of *Haemogregarina nucleobisecans* from *Bufo melanostictus* and *Bufo andersoni*
- | | |
|--|--------|
| Fig. 1. Gametocytes in the peripheral blood of <i>B. melanostictus</i> | X 550 |
| Fig. 2 & 3. Elongate mature gametocytes in the blood of <i>B. melanostictus</i> | X 1665 |
| Fig. 4 & 6. Elongate mature gametocytes of <i>H. nucleobisecans</i> in <i>Bufo andersoni</i> | X 1400 |
| Fig. 7. A mononucleated schizont within the liver tissue of <i>B. melanostictus</i> | X 1110 |
| Fig. 8. A mature schizont | X 1400 |
| Fig. 9. A multinucleated mature schizont within the liver parenchyma | X 1400 |
| Fig. 10. A mature schizont with developing merozoites | X 1665 |

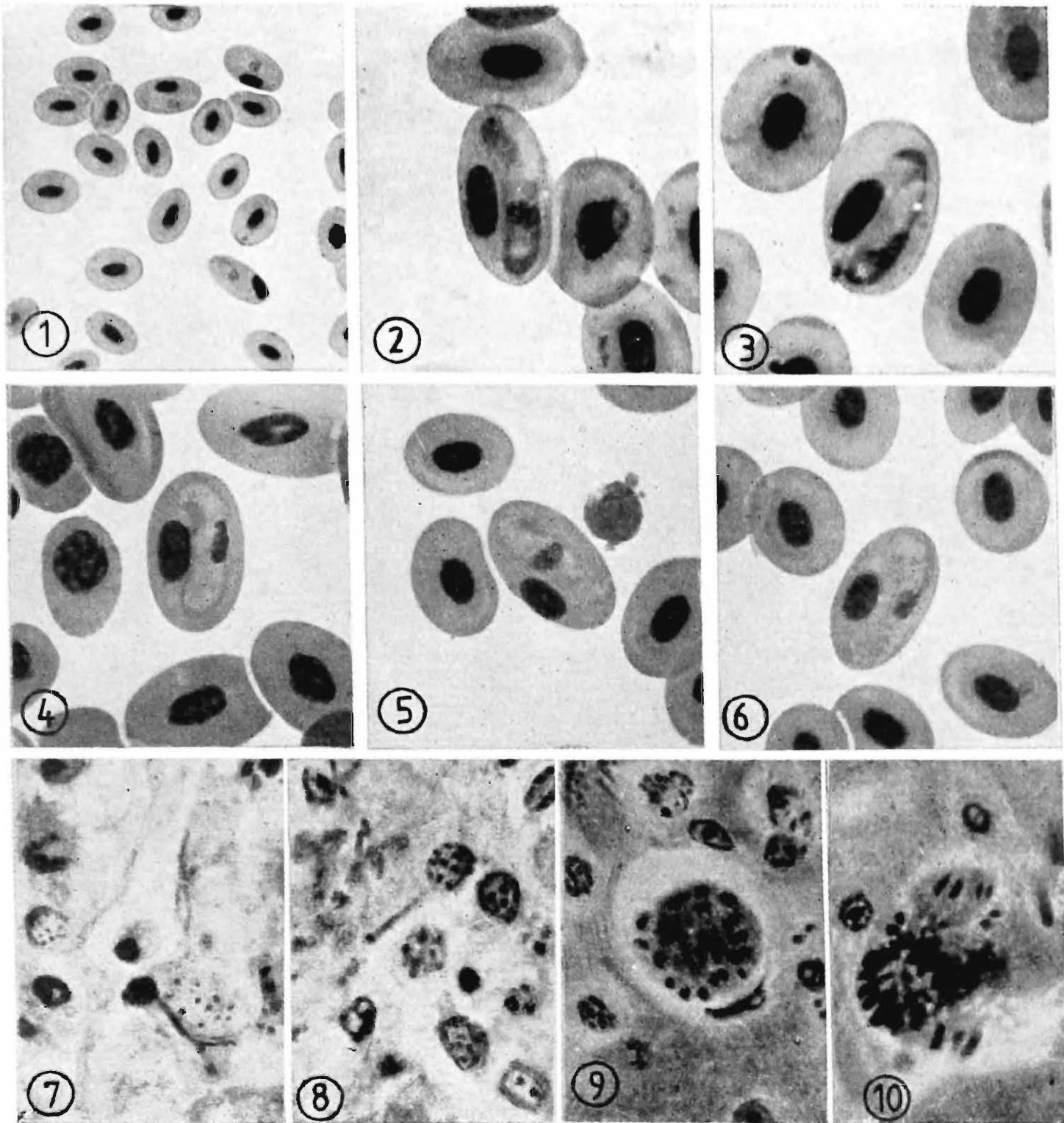


PLATE IV

- Figs. 1-4. Gametocytes of *Haemogregarina perinucleophilum* in the blood of *Rana tigrina*.
Figs. 1 & 2. Microgametocytes X 3550
Figs. 3 & 4. Macrogametocytes X 2165
- Figs. 5-8. Gametocytes of *Haemogregarina maculatus* sp. nov. from *Rhacophorus maculatus*
Fig. 5. Elongate mature macrogametocyte with anterior beak-like (b) structure. X 1665
Fig. 6. Microgametocyte X 1570
Fig. 7. Macrogametocyte with posterior plug-like (P) structure X 1665
Fig. 8. Hypertrophied erythrocyte with double infection X 1560
- Figs. 9-13. Gametocytes of *Haemogregarina pattoni* sp. nov. from *Rana hexadactyla*
Fig. 9. Immature gametocyte with capsular band (Arrow) X 2000
Fig. 10. A mature "Capped" macrogametocyte. Arrow indicates the cap. X 1507
Fig. 11 & 12. Microgametocytes showing parenthetical capsular band. X 1500, X 1800
Fig. 13. Extracorpuseular free gametocyte within a capsule (ca). X 1500

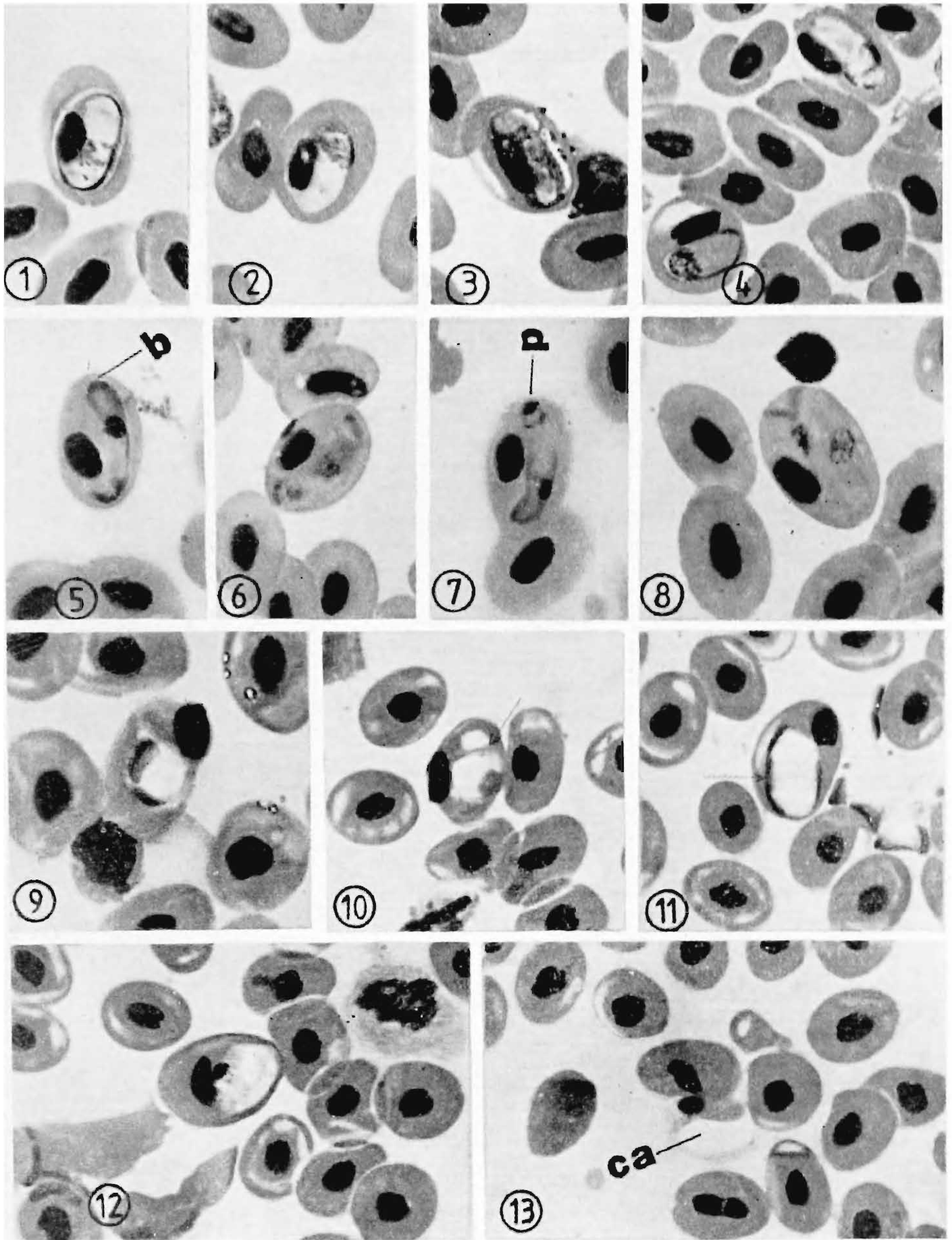


PLATE V

- Figs. 1-8. Gametocytes and tissue stages of *Haemogregarina kaloulae* sp. nov. from *Kaloula pulchra taprobanica*
- | | |
|---|--------|
| Fig. 1. Gametocytes in the peripheral blood | X 515 |
| Fig. 2-4. Elongate mature gametocytes | X 1550 |
| Fig. 5. Erythrocytic binucleated schizont | X 1250 |
| Fig. 6. Extracorpuseular free gametocyte | X 1935 |
| Fig. 7. A mature schizont in the liver tissue by the side of central vein (CV). | X 1835 |
| Fig. 8. Arrow shows the released merozoites | X 1000 |
- Figs. 9-11 Gametocytes of *Haemogregarina andamanensis* sp. nov. from *Rana cyanophlyctis*.
- | | |
|--|--------|
| Figs. 9 & 10. Elongate mature gametocytes | X 1175 |
| Fig. 11. Hypertrophied erythrocyte with double infection | X 1175 |
- Fig. 12. Gametocyte of *Haemogregarina pulchra* sp. nov. from *Kaloula pulchra*.
- | | |
|--|--------|
| | X 1200 |
|--|--------|
- Fig. 13 & 14. Gametocytes of *Haemogregarina systoma* sp. nov. from *Uperodon systoma*
- | | |
|--|--------|
| | X 1250 |
|--|--------|

