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ON THE OCCURRENCF OF HAEMOGREGARINA NUCLEOBISECANS SHORTT, 1917 IN TOADS OF WEST BENGAL

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ABSTRACT

Haemogregarina nucleobisecans was found to infect two species of toad *viz.*, *Bufo melanostictus* (13.4%) and *B. andersoni* (28%) from Bankura and Purulia districts, West Bengal, India. Intra-erythrocytic development and tissue schizogony of the parasite in the liver parenchyma from both the bufonid hosts were described and illustrated. *H. nucleobisecans* has been recorded from a new geographical locale of Eastern India and from a new host *Bufo andersoni*.

Introduction

Shortt (1917) described *Haemogregarina nucleobisecans* for the first time from *Bufo melanostictus* collected from Cawnpore, Delhi and Ambala (Punjab), India. Subsequently, Wenyon (1926) reported the occurrence of this parasite from the same host in India. Since then there was no information on aphibian haemogregarines till the reports of Ray (1977, 1979). Sinha (1979) reported one *Haemogregarina* sp. from *Bufo himalayanus* of Darjeeling, West Bengal.

The present paper deals with the detailed description of *Haemogregarina nucleobisecans* in two species of Toad *viz.*, *Bufo melanostictus* and *B. andersoni* along with the schizogony in the liver of both the hosts. *Bufo andersoni* has been recorded as a new host for this haemogregarine parasite.

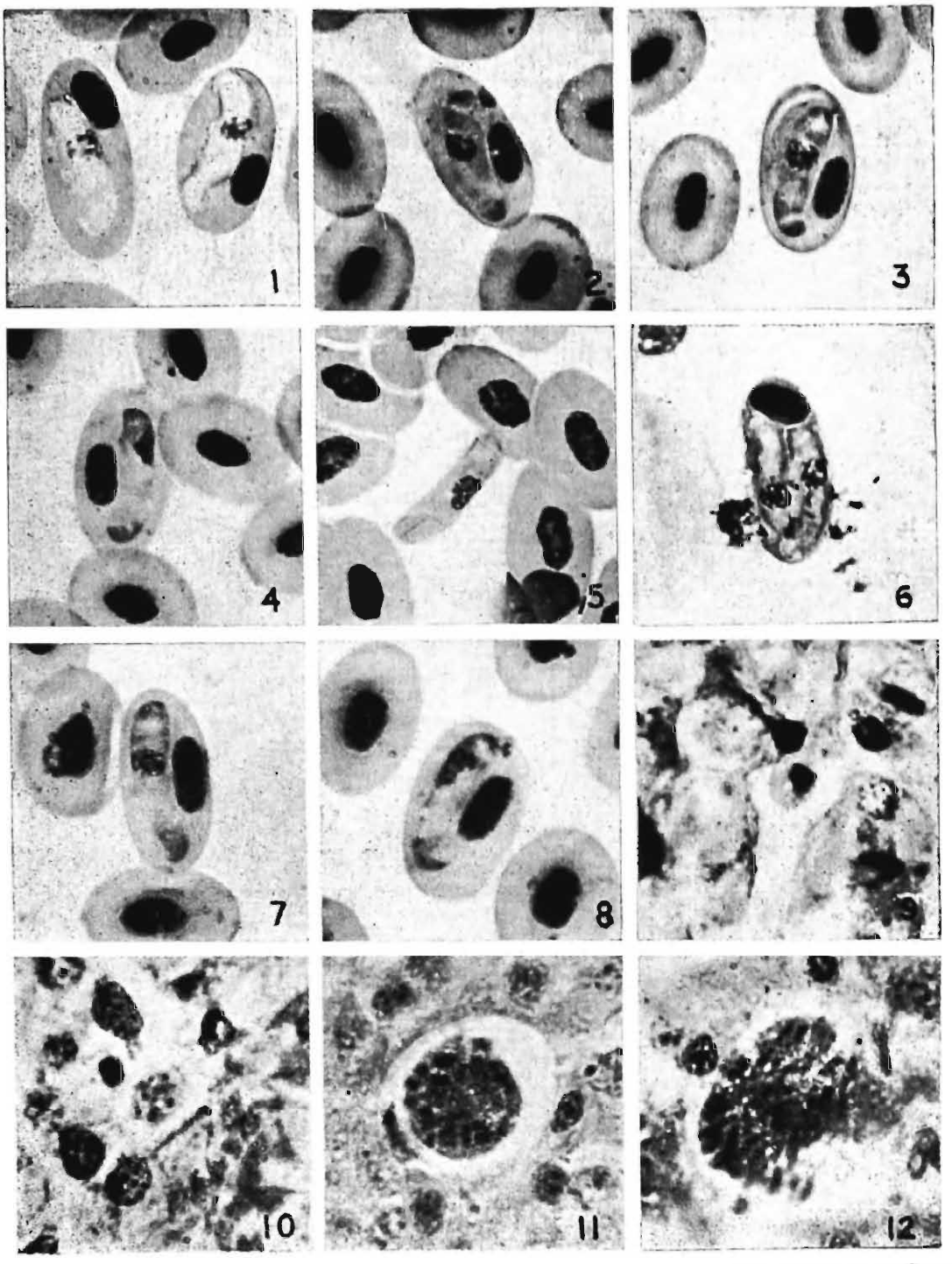
Material and Methods

Both the hosts were collected from Bankura and Purulia districts of West Bengal and some of them were kept alive in the laboratory for examination. Peripheral blood was obtained from finger tips on alternate days and at autopsy. Impression and spread preparations were made from liver, lungs, kidney etc. Air-dried blood films and organ imprints were fixed in 100% methanol and stained with Ramanowsky type of stains. For microtome section the tissue of lung, liver, kidney and spleen were fixed in alcoholic Bouin's fixative, followed by general histological technique (Pearse, 1960) and stained with iron-haematoxylin and eosin.

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

Observation

47 13.4% out of 343 examples of *Bufo melanostictus* and 7 (28%) out of 25 *B. andersoni* examined were found positive for the parasite. A minimum of 5% to a maximum of 15% of the total R. B. C. counted were found to be infected with *H. nucleobisecans*. The description of the parasite is as follows :



Figs. 1-12. Photomicrographs of the erythrocytic and tissue stages of *Haemogregarina nucleobisecans* from *Bufo melanostictus* and *Bufo andersoni*.

1-4.	Elongate mature gametocytes in the perepheral blood of *B. melanostictus*.	X 1665
5.	An extra-corpuscular free gametocyte	X 1800
6.	An erythrocyte with double infection	X 1665
7-8.	Elongate mature gametocytes in *Bufo andersoni*.	X 1800
9.	A mononucleated schizont within the liver tissue.	X 1110
10.	An immature schizont	X 1400
11.	A multinucleated mature schizont within the liver parenchyma.	X 1140
12.	A mature schizont with developing merozoites.	X 1665

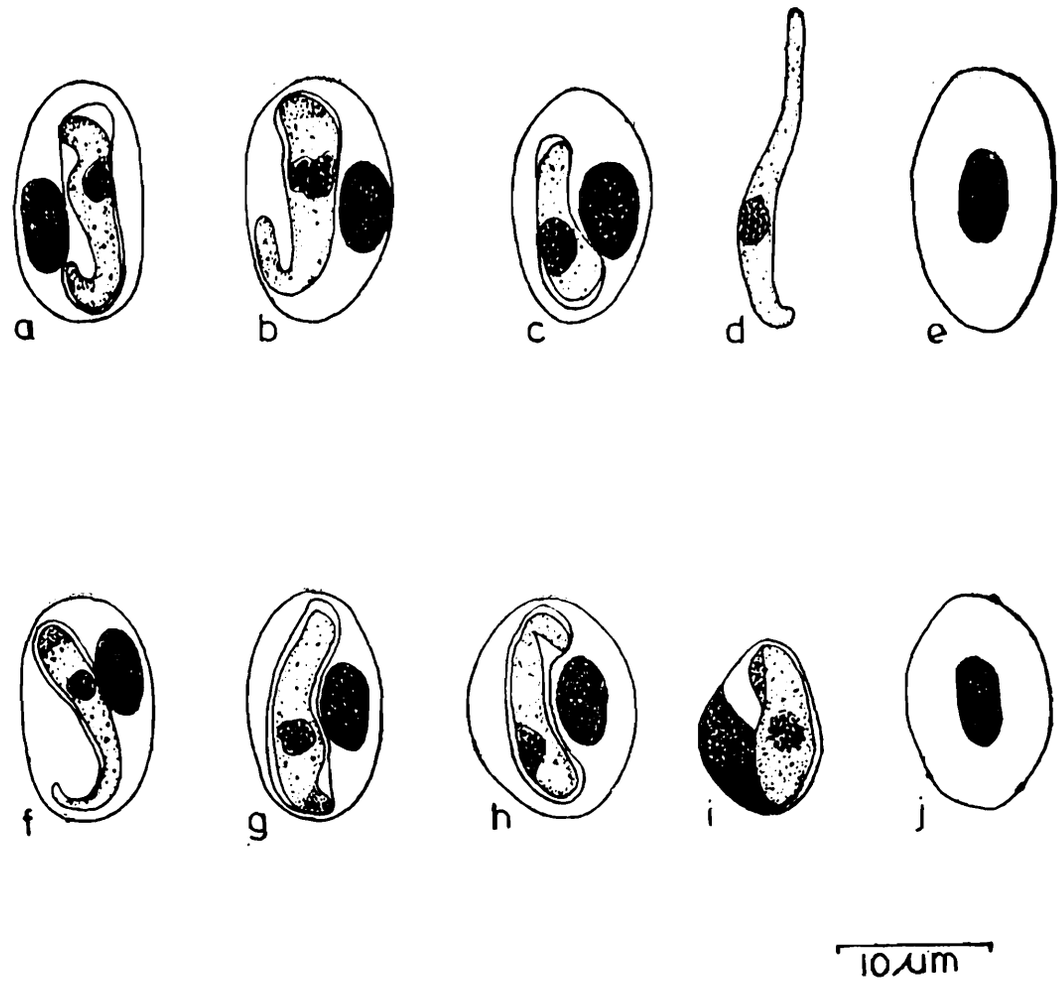


Fig. 1. Camera-lucida drawings of the erythrocytic stages of *Haemogregarina nucleobisecans* from two anuran hosts.

a-d, the gametocyte stages of *H. nucleobisecans* in *Bufo melanostictus*. a & b, the elongate mature forms ; c, the young forms ; 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.

Gametocytes :

Elongate mature (Figs. 1a-c & Pl. V Figs. 1-4) :

N=15. These are sausage shaped endoparasities of erythrocytes, slightly curved and lying with its concave border applied to the convexity of the host cell nucleus or *vise-versa*. The anterior end is regular, rounded and broad while the posterior end is usually slightly bent (Fig. 1 a) or hook-like (Fig. 1 b). In some cases both the extremities may be recurved and afflicted or the parasite may show a constriction about its centre. These elongate forms measure 14.4 μm × 2.95 μm with an average area of 28.95 μm^2 occupying about 25.97% of the total host cell-parasite complex. Granulated cytoplasm stains azure blue or faint pink with Giemsa stain. The nucleus, approximately central in position, almost fills up the entire width of the parasite. It may be rounded (Fig. 1 f), oval (Fig. la) or band-shaped or irregular in outline, measuring 3.25 μm x 1.95 μm and 5.18 μm^2 in area ; stains pink with Giemsa. The

gametocytes are always enveloped in a pink coloured thick capsule.

Intermediate and young forms (Fig. 1 c) :

Intermediate forms measure 10.5 μm×3.0 μm and are comparatively rare in the peripheral circulation. Cytoplasm stains light blue and contains a few metachromatic granules. The nucleus is the aggregation of chromatin materials at the centre of the parasite. Young forms measure 5.2 μm×2.5 μm ; both the ends rounded, capsule-like ; band-shaped nucleus at the centre. Cytoplasm stains deep blue, with very few metachromatic granules.

Extracorpuscular free forms (Fig. 1 d & Pl. V, Fig. 5) :

These are elongated with one end rounded and the opposite end being attenuated ; measuring 21.72 μm×2.84 μm with 24.8 μm^2 in area. The cytoplasm is bluish with metachromatic granules evenly distributed. The compact rounded nucleus measures 3.2 μm× 2.28 μm with an area of 4.9 μm^2.

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 was placed peripherally or sometimes pushed to apical position in case of double infection (Fig. 6). Nuclear Displacement Ratio=0.17. Host cell hypertrophied in length (7.3%) and area (11.3%). Host cell nucleus also hypertrophied in area (17%).

Uninfected erythrocyte :

N=15. Cell 15.47 μm×8.55 μm and 100.10 μm^2 in area. Cell nucleus 5.85 μm× 2.72 μm and 13.28 μm^2 in area. N. D. R.= 0.97.

Schizogony (Pl. V, Figs. 9-12) :

A thorough examination revealed some schizogonic stages in the liver parenchymatic cells. Erythrocytic schizogony was not detected. The early developmental stage of a schizont is an oblong or spherical uninucleate body measuring 4.5×4.2 μm ; occupying a cavity (Parasitophorous vacuole) (Fig. 9) within the liver cell. The spherical nucleus is 2.5 μm in diameter, surrounded by an areola. The multinucleate schizonts (Fig. 11) measure 15.00 μm×14.00 μm. The larger mature schizonts measure 20 μm×13 μm and contain many slender curved merozoites (4.5 μm×1.5 μm).

A more or less spherical nucleus is situated at the centre of each merozoite which stains deep black with Iron alum haematoxylin stain.

Discussion

Haemogregarina nucleobisecans along with its hepatic schizogony was first reported by Shortt (1917) from North 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 respects. The bisecting nature of the host-cell nucleus, however, has not been observed by the present author. Shortt (1917) did not observe any movement of this parasite but the present author noted a characteristic slow movement of extracorpuscular gametocytes in the citrated saline preparation of fresh blood.

H. nucleobisecans has also been recorded during the present investigation from *Bufo andersoni* which is little smaller than that described from the type host. Moreover, in *B. andersoni* some leucocytes were also found

to be infected with the parasites measuring 11.5 μm×2.5 μm.

Tissue Schizogony of the parasite has been observed in both the hosts *Bufo melanostictus* and *B. andersoni*. One type of schizogony with only one type of merozoite was noted.

Haemogregarina nucleobisecans has been recorded from a new geographical locale of Eastern India and from a new host *Bufo andersoni* along with *B. melanostictus*.

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