

HAEMOPROTEUS OVALIS SP. NOV. (PROTOZOA : SPOROZOA) FROM AN INDIAN
PADDY-FIELD FROG, *RANA LIMNOCHARIS* WIEGMANN

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ABSTRACT

Haemoproteus ovalis sp. nov. is described from an Indian Paddy-field frog *Rana limnocharis* from Balitha, Bankura district, West Bengal, for the first time in India. The parasite shows sexual dimorphism. Its affinities with related species is discussed.

INTRODUCTION

The genus *Haemoproteus* is characterised by pigmented halter-shaped gametocyte in erythrocytes of the circulating blood. The haemoproteid parasites are fairly common in wild and domestic birds and also found in some cold-blooded vertebrates like reptiles and amphibians.

The first member of the genus *Haemoproteus* in Amphibia was described by Fantham *et al.*, (1942). They described three species of *Haemoproteus* viz., *H. laurentae*, *H. lavalia* and *H. lanorailla* in *Bufo americanus* from 3 different localities of eastern Canada. Levine and Campbell (1971) listed the name of the above mentioned three species of *Haemoproteus* of Amphibia in their "Check-list of the species of the genus *Haemoproteus* (Apicomplexa, Plasmodiidae)".

While surveying the blood parasites of Indian Amphibians (Ray, 1979) the authors observed one *Haemoproteus* in the blood of

Rana limnocharis along with a mixed infection of *Haemogregarina berestneffi* Castellani and Willey and described in this present communication for the first time in India.

The type specimens will be deposited in the National Collection of the Zoological Survey of India, Calcutta.

MATERIAL AND METHODS

The frogs were collected from a village viz., Balitha in the Bankura district, West Bengal and brought to the laboratory for examination. The blood smears were generally prepared from the blood obtaining by cutting the finger and toe tips. Some smears were also prepared from lungs, liver, spleen, kidney and heart of the infected frogs.

All smears were air-dried, fixed in 100% methanol, stained with Romanowsky stains and differentiated with neutral distilled water. For sectioning the tissue of lung, liver, kidney

and spleen were fixed in Bouin's fixative and followed by general histological techniques by Pearse (1960). The sections were stained with iron-haematoxylin and eosin.

Each smear was examined for 5 minutes under low magnification ($450\times$) and subsequently under oil immersion ($1000\times$). 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 morphometric parameters were measured after Bennett and Campbell (1972). The photomicrographs were taken with the help of 'Ergavel' C.Z. microscope using PM6 attachment Camera.

OBSERVATIONS

Haemoproteus ovalis sp. nov.

(Pl. IV, figs. 1 & 2 ; Fig. 1, a-c)

Description : *Immature gametocyte* : (Pl. IV, fig. 1 ; Fig. 1a) N=15. These are intracorpous rounded to oval parasites, measuring $6\ \mu\text{m}$ in length and μm in width with a

granules (Approx. 10) in the form of small dots or rods, found to be distributed throughout the cytoplasm. Sometimes a small vacuole may be seen at the periphery. The nucleus is rounded to oval, measuring $2\ \mu\text{m}$ in diameter with a mean area about $2.5\ \mu\text{m}^2$ and stained light pink with Leishman and Giemsa. These parasites are situated in close contact with the host cell nucleus at the periphery.

Microgametocyte (Fig. 1b) : N=15. These are elongated pyriform parasite, measuring $8\ \mu\text{m}$ in length and $4\ \mu\text{m}$ in width with an average area of $22.0\ \mu\text{m}^2$ and occupy 16.76% of the total host-cell parasite complex. They are situated at one end of the gametocyte or sometimes it pushes the host-cell nucleus at one corner. Cytoplasm is homogeneous, hyaline ; stained light blue with Leishman, finely granulated. The yellowish black pigment granules are about 7 in number, either in the form of small dots or rods and distributed throughout the cytoplasm. The nucleus is fragmented and less compact, measuring $1.8\ \mu\text{m}$ in diameter and sometimes it is not well marked. A small vacuole may

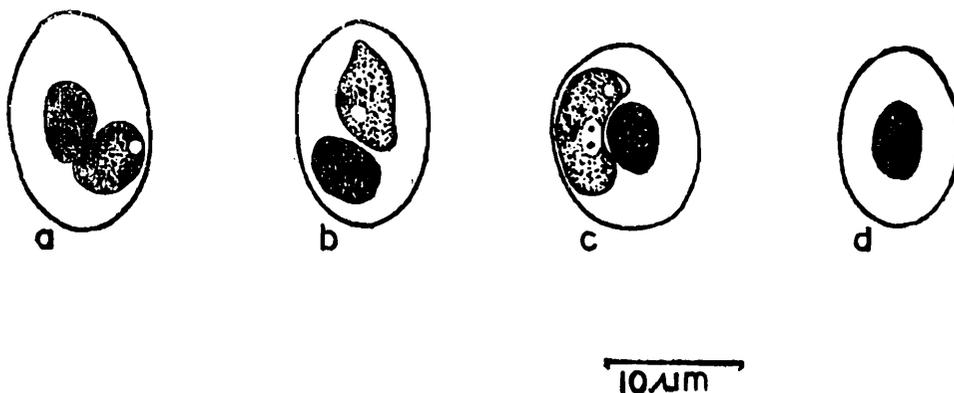


Fig. 1 (a-d). *Haemoproteus ovalis* sp. nov. in *Rana limnocharis* :
a, Immature gametocyte ; b, microgametocyte ; c, macrogametocyte ;
d, uninfected erythrocyte.

mean area about $16.5\ \mu\text{m}^2$, occupying 13.89% of the total host cell-parasite complex. The cytoplasm is homogeneous, stained light blue with Leishman Giemsa. A few pigment

be present. It stained very faint purple with Leishman.

Macrogametocyte : (Pl. IV, fig. 2 ; Fig. 1c) N=15. Bean or kidney shaped parasites,

measuring 9 μm in length and 4 μm in width with a mean area about 23.5 μm^2 and occupying 20.88% of the total host-cell-parasite complex. These are situated at one side of the R. B. C. facing its concave side towards the convex margin of the host cell nucleus. The cytoplasm is homogeneous, a little granular and stained deep blue with Leishman and Giemsa stains. Pigment granules are approximately 10 in number and oriented in the same manner as in male gametocytes. They are yellowish black in colour and sometimes 2 or 3 of them congregate to form a bigger mass. One vacuole may be seen in some cases.

Uninfected erythrocyte : (Fig. 1d) N=15. Cell 12.9 μm by 9.1 μm and 86.97 μm^2 in area. Cell nucleus 5.3 μm by 3.3 μm and 13.04 μm^2 in area. NDR=0.95.

Tissue stages and vector is unknown.

Occurrence : Out of 307 examples of *Rana limnocharis* examined, only 2 were found to be infected with this *Haemoproteus* parasite.

Type material : Holotype (Z. S. I. Reg. No. 1951) is designated to a blood smear taken from *Rana limnocharis* Wiegmann from Balitha, Bankura district, West Bengal, India, on 1.7.1977, collected by Sri R. Ray. Paratype (Z. S. I. Reg. No. 1952) collection data same as holotype.

DISCUSSION

A perusal of the literature on Amphibian haematozoa reveals that there is no substantial record of *Haemoproteus* parasites in this craniate class except that of Fantham *et al.*, (1942) who reported 3 species of this genus viz., *H. laurentiae*, *H. lavalia* and *H. lanoraila*

TABLE—1

Parasite and reference	Gametocyte		Nucleus		Host
	Length in μm	Width in μm	Length in μm	Width in μm	
<i>Haemoproteus ovalis</i> sp. nov.					<i>Rana limnocharis</i>
Macrogametocyte	9.0	4.0	2.5	2.0	
Microgametocyte	8.0	4.0	1.8 μm in diameter		
<i>H. laurentiae</i> Fantham <i>et al.</i> 1942	10.1-17.7	3.0-7.2	—	—	<i>Bufo americanus</i>
<i>H. lavalia</i> Fantham <i>et al.</i> 1942	10.8-16.2	5.2-7.8	3.9-6.2	3.0-4.5	<i>Bufo americanus</i>
<i>H. lanoraila</i> Fantham <i>et al.</i> 1942	11.8-16.7	4.4-8.5	3.0-5.2	2.2-3.7	<i>Bufo americanus</i>

Effect of the parasite on their host cell : Infected erythrocytes were little hypertrophied. The host cell-nucleus became distorted and displaced at one pole of the erythrocyte.

from a single host *Bufo americanus* of Eastern Canada.

As no report on *Haemoproteus* is in existence from the family Ranidae in the world, the

authors enjoy the credit to add the fourth *Haemoproteus* species from a Ranid frog, to the literature concerning Amphibian haematozoa.

Among the three *Haemoproteus* parasites described by Fantham *et al.*, (1942), only *H. lavalia* comes to proximity with the *Haemoproteus* species dealt in the present communication. But a comparison of the mesural data of all the four parasites (Table 1), Canadian and Indian, would lead to the assertion that the parasite described from the Indian frog, *Rana limnocharis* would emerge as a distinct species. The authors are able to differentiate the macro- and microgametocytes in the species under discussion in contrast to the observations made by Fantham *et al.*, (1942) where no evidence of sexual dimorphism in any of their *Haemoproteus* parasites could be forwarded.

Judgement of the above contentions leads the authors to declare the present parasite as a new species and hence the name *Haemoproteus ovalis* sp. nov. has been coined after the parasite's body configuration.

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