

# A CONTRIBUTION TO THE STUDY OF THE BLOOD PARASITES OF SOME INDIAN BIRDS.

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## 1. Introduction.

IN these notes a short description will be given on the blood parasites found in some Indian birds which eventually came to my hands. The work is not a systematic one as owing to many other official duties I can but spare very little time to such investigations.

There is one point which I am wishing to insist upon: it concerns the nomenclature of the species of the parasites found in birds. As far as concerns *Hæmoproteus*, no proofs have been given that one and the same species of *Hæmoproteus* may occur in different birds. Secondly, the morphological data and the tinctorial reactions are sometimes so different in the *Hæmoproteus* of the different birds that no morphologist would have any doubt to name the species, at least, according to the host in which it occurs. So, in this work the species will be named according to the host and if the morphological elements are clear enough to establish more than one species, the second one will have another name.

As far as concerns the *soidisant* bird malaria: the designation *Proteosoma* is definitely discarded in this work, as neither zoological nor genetic reasons plead for keeping this generic name. The writer does not agree with Doflein<sup>1</sup> and others who still claim for retaining this designation, neither that all the species found in birds may belong to one single species, the so-called *Proteosoma precox*. No doubt that one strain from sparrows, for instance, can be inoculated to canaries, larks, goldfinches, etc., but if in the canary itself Hartmann is able, on morphological grounds, to differentiate three species of *Plasmodium*<sup>2</sup> and Russel<sup>3</sup> a fourth one, it is not logical that we should identify all species of birds Plasmodia having a remote appearance with *P. precox* as a true *Proteosoma precox*, unless definite proof through inoculation, etc., be obtained that a given plasmodid of a bird is not really but the *P. precox* of canaries and sparrows. So, in this work the plasmodids will be also named according to the host and when the appearance is much alike to the cycle of *P. precox*, this similarity will be noted.

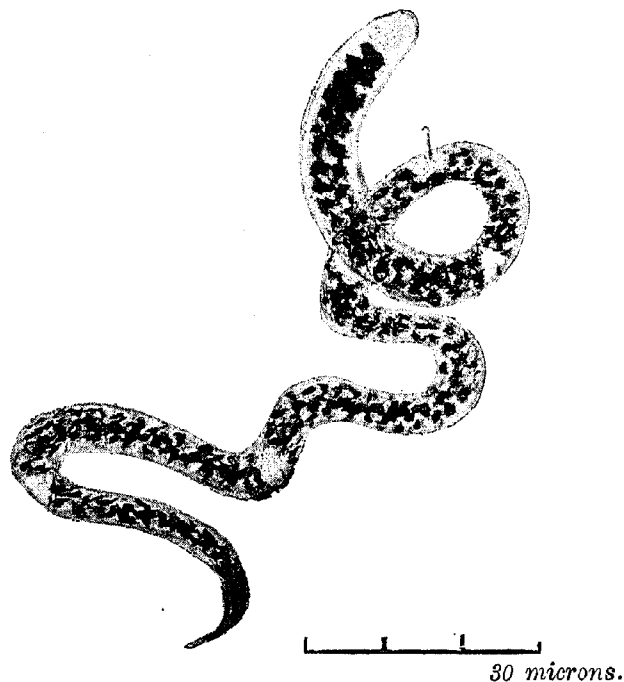
Whenever possible, specially when the infection is a heavy one, attempts will be made to work on the schizogonic cycles, which will probably constitute an object of special papers.

The list of the blood parasites joint to the book of Wenyon<sup>4</sup> has been to me of great help.

The films have been made after shooting the bird, and prepared sometimes *in loco*, sometimes three to four hours after. The films have been stained by Romanowsky (Leishmann, May-Grunwald-Giemsa), in special cases by other methods which will be quoted in every case. The illustrations have been prepared always from Leishmann stained films in order to put into evidence the morphological and tinctorial differences seen in the slides.

#### I. *Herodias intermedius* Wagler.

This heron (1 specimen) has been shot in the lake of Carambolim, Department of Ilhas Goa, and identified by our colleague in the Academy, Prof. Narayan Rao, from the Zoological Department of the Central College, Bangalore, to whom my best thanks are due.



Microfilaria of *Herodias intermedius*.

Slides from blood of the heart and smears of the lungs, stained by Leishmann stain and panoptic method. As far as I am aware, among the birds of the genus *Herodias*, only *H. alba* has been examined on this point of view in Belgian Congo by Rodhain, Pons, Vandenbranden and Bequaert (1913) who registered a *Hæmoproteus* in the blood of this congolese heron.

Our specimen gave the following parasites:—

(a) A *Giardia*, in very scanty number. Under the name *Giardia sanguinis* Gonder (quoted in Wenyon, Doflein) described this flagellate in the blood films of a falcon shot in Transvaal. Wenyon<sup>4</sup> thinks that it was due to an intestinal contamination. As the intestines were not touched by me when making the blood films, I am rather inclined to suppose that there was perhaps a migration of the intestinal flagellates to the blood after the animal was shot. The intestines were full of *Giardia*, which will be described in a special paper.

(b) A *Plasmodium* which I will name *Plasmodium herodiadis* n. sp. The infection is not very heavy. The small schizont has a ring form with the chromatic dot either granular or rod-like. Some forms in a little more advanced stage of development have a pyriform appearance, with one pointed end. The grown-up schizonte or trophozoite is roundish or aberrant and does not occupy more than one quarter of the red cell. It is full of pigment granules of different sizes and irregularly scattered, the colour of the pigment being brownish black. The chromatin is either a grain or a batonnet, this last form seeming the first stage of division. Rosettes with five or ten chromatinic bodies, the complete formation of merozoites having not been found. Gametes roundish, pigmented, with round nucleus. Some gametes somewhat oval, with a strong, irregular nucleus are interpreted as male gametocytes. This plasmodid is perfectly distinguishable from the *Hæmoproteus* which is found in the same blood, not only by its form, but specially because the nucleus is always stained in vivid red whilst that of *Hæmoproteus* shows a pale rose colour. The red cells are not altered.

(c) A *Hæmoproteus*, which I will name *Hæmoproteus herodiadis* sp. n.(?) and is perhaps similar to that found in the *Herodias alba* by the Belgian authors. Parasites were found in greater number than the former ones. Sexual dimorphism of the gametocytes characterised only by the tinctorial reaction of the protoplasm to Romanowsky: whilst light blue, alveolar in females, it is colourless, almost white in males. No sexual difference on the distribution of the pigment, as it happens in so many species of this genus. Here, the pigment is irregular in form, in the size of granules and its distribution, either in males or in females. The colour of the pigment is brown black.

Concerning the form of the gametocytes, it is roundish oval in young stages tending to an incomplete halteride, with regular borders, rarely attaining such a grown-up halteride which embraces the nucleus of the red cell as in many other species. It is not rare to see fusiform or irregularly bent forms, such irregularities being seen specially in female gametocytes.

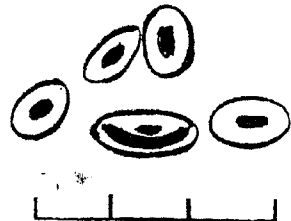
One thing that I have remarked in this species is that in the protoplasm of the female halteride there are sometimes small rings, violet coloured, isolated or in clusters which I have not seen in any other species I have worked with. Their violet staining contrasts with the pale rose colour of the nucleus. And what is more curious is that such rings I have detected too in the protoplasm itself of the red cell. One should be inclined to consider such rings as *rings of Cabot*, but their presence in the parasite leaves me very reserved on their nature.

No schizogonic form was seen in the smears of organs, but smears are not, *d'ailleurs*, the proper means for such study. The host cells are not altered.

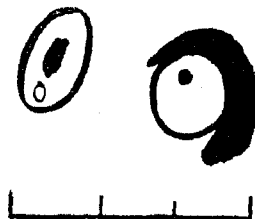
(d) A *Microfilarium*.—The infection was so rare that only *one* specimen was discovered by chance in a blood smear. Provided with sheet, the nuclei beginning at 8 microns from the anterior end, rounded. Posterior end pointed. Four spots, the median one somewhat in V form. Largest breadth 10 microns. The figure gives an exact idea of the parasite.

## II. *Gallinula chloropus* Linn.

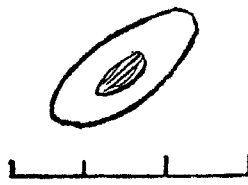
One specimen, shot in the lake of Carambolim. Identified by Prof. Narayan Rao. The blood of this bird has given to Coles (1914) in England (quoted by Wenyon) a *Leucocytozoon*. We have not found it in our specimen



30 microns.  
Hæmoproteus of *Machlolophus xanthogenys*.



30 microns.  
Plasmodium and Leucocytozoon of *Chloropsis aurifrons davidsoni*.



30 microns.  
Red cell of *Herodias intermedius*.



30 microns.  
Plasmodium of *Gallinula chloropus*.

but a *Plasmodium*, which we will name *Plasmodium gallinulæ* n. sp. and we will describe now.

Infection very scanty. Intraglobular forms very rare and seen only in young stages, which are annular, under the form of very regular rings. If some of them are with a slight trace of blue protoplasm as in young rings of *Pl. falciparum*, others, probably in a further stage of development, show the protoplasm more deeply and extensively blue. Will it be the first stage of a gametocyte? It cannot be definitely decided. The schizont at a further stage is oval and of increased size. The infected red cell is hypertrophied and, I suppose, that completely lysed afterwards, in spite of having not seen such figures. The large pigmented trophozoite of aberrant ameboid form which is shown in the illustration seems to me provenient of the supposed desagregation of the red cell. The pigment is brown-black, brownish or almost black.

The gametocytes are formed inside the red globules. They are the main plasmodid stages seen in our preparations and show a clear sexual dimorphism. The female gametocytes stain in a compact and deep blue, have a large nucleus, the pigment granules are rather in larger dots, the male gametocytes are roundish or oval, alveolar, the nucleus constituted by many chromatic rods irregularly connected, the pigment granules smaller and more or less scattered through the protoplasm.

I was not able to detect any divisional stage either in blood films or in organ smears. The morphological appearance of this plasmodid is very different from that of *P. precox* of Canaries.

### III. *Machlolophus xanthogenys* (Vigors).

Plimmer in 1912 recorded a *Hæmoproteus* in the blood of this Indian bird.

Our specimen, shot at the village of Siroda, of the Department of Pondá, has been identified by Dr. Baini Prashad from the Zoological Survey, Indian Museum, and to him my best thanks for the help he so many times has given me.

A *Hæmoproteus* has been also seen in our preparations. Infection not very abundant. The infected cell is hypertrophied, the nucleus not displaced. Sexual dimorphism of the gametocytes constituted by the tinctorial reactions of the protoplasm which is, with Leishmann stain, grayish blue in the female and clear, almost white in the male. The pigment is constituted by rather minute dark brownish granules not very abundant irregularly scattered over the protoplasm in the female, showing a tendency to collect in the poles in the male. The outline of the female is very

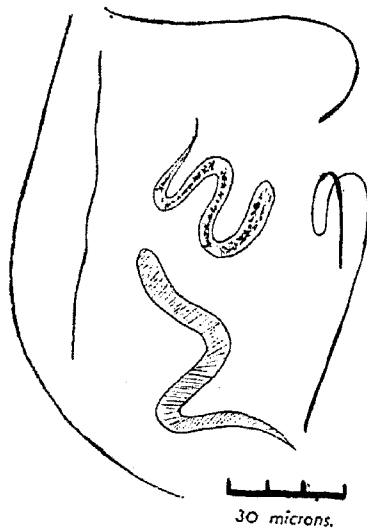
irregular, denticulated as it can be seen in the figures whilst in males the lines are regular. Haltherid forms not very conspicuous as in other species of this genus. Nucleus pale rose, subcentral.

Moreover in the general morphology which we have described we insist on the greyish blue tone of the female protoplasm as one of the characteristic of this species.

We are convinced that this *Hæmoproteus* is the same as that studied by Plimmer. Should it not have been named, we will baptise it under the name *Hæmoproteus machlolophi*.

#### IV. *Chloropsis aurifrons davidsoni* Baker.

The list of Wenyon gives *Chloropsis* (sic) *aurifrons* from India as harbouring *Plasmodium precox* and *Hæmoproteus* according to Dr. H. H. Scott. I think that it is the same bird and *Chloropsis* a typographic mistake.



Microfilaria of *Chloropsis aurifrons davidsoni*.

Our specimen identified by Dr. Bains Prashad was shot at Mardol, Department of Pondá and did not show any *Hæmoproteus*, but the following parasites:

(a) A *Plasmodium*.—Young schizonts annular resembling those of *Plasmodium falciparum*. Larger schizonts under the form of big pigmented rings, the pigment granules being of brownish colour. Rosettes with (or without) pigment, situated generally on the periphery, varying number of merozoites going from 6 to 11 in our slides. Male gametocytes oval with large irregular nucleus. Female gametocytes roundish, generally very deeply stained in blue with round or oval, rod-like, nucleus. Red corpuscle not altered.

I believe that this *Plasmodium* is the same as that registered by Dr. H. H. Scott as *P. precox*, but I do not agree with such identification. Although having a general resemblance with *P. precox* specially as far as concerns the multiplication stages and the irregular number of merozoites, the nucleus of these merozoites is totally different in its compact structure from the ring nucleus of the *precox* merozoites. The oval male gametocytes are also to be considered.

I will describe further on the morphology of *P. precox* as it is found in canaries and sparrows in India, to render more evident that the species parasite of *Chloropsis* cannot be identified with *P. precox*, at least on morphological grounds.

I will name it *Plasmodium chloropsidis* (Scott, 1925) mihi 1934.

(b) A *Leucocytozoon* from which I can at present only record the species, hoping that with further material I will be able to say something about the life cycle of this genus—a problem yet unsolved and one of the most fascinating among those connected with Hæmoprotozoa.

The name *Leucocytozoon* was given by Danilewsky<sup>5</sup> for certain unpigmented parasites of the blood of birds which, he stated, were included "non dans une hematie, mais dans un leucocyte en état de dégénérescence". They have been found in many birds and generally under two types: one, contained in cells with elongated fusiform points, the other in roundish cells whose nucleus encircles more or less the parasite. Léger<sup>6</sup> has suggested that it would be convenient to designate these two forms under two generic different names as "la cellule hôte est dans certain cas un hémotoblaste, dans d'autres cas un mononucléaire, jamais indifféremment l'un ou l'autre." As regards the nature of the host cell the opinions are also very different. Mathis et Léger<sup>7</sup> claim for the following formula: host cell fusiform=erythroblast; host cell roundish=mononuclear leucocyte. In the final note of their paper they state: "distinguer deux catégories de Leucocytozoon suivant la nature de la cellule hôte parasitée, ce serait à notre avis, prématuré." One must not forget that Wenyon<sup>4</sup> has written: "Working with *L. neavei*, the writer noted that whereas in fresh blood preparations all the parasites were in spindle-shaped cells, in dried films, especially if made some time after the date of the bird, there was a much greater diversity of shape, many of the parasites being spherical, while the cells appeared to have lost their tail-like prolongation."

Mathis et Léger<sup>7</sup> say that Danilewsky who at first thought that the host cells were degenerated leucocytes, later on considered them *hematoblasts*. Their opinion is followed by Sambon. Wenyon, Keysselitz and

Mayer classify such cells as erythroblasts. Laveran and Lucet believe that the L. of the Turkey is included in white corpuscle, Woodcok thinks that *L. fringillinarum* infects a mononuclear. França<sup>6</sup> classifies the host cell of *L. laverani* par. of *Garrulus gleodarius* L. as a mononuclear leucocyte, that of the young forms of *L. mathisi* par. of *Accipiter nisus* as an hematia. In another paper<sup>8</sup> França says that the nature of the infected cell can be determined only when the parasite is very young "parce que les L. altèrent de bonne heure et profondément la nature de la cellule hôte. Ce n'est pas la forme de la cellule hôte qui determine la configuration du parasite, mais bien la forme de celui-ci qui cause celle de la cellule hôte." He described also some pigmented forms, the nature of this so-called pigment being denied by Léger<sup>9</sup> in *L. zucarellii* par. of *Corvus corone*, where the granules should be identified with the "acidophil granulations" described by André Léger et P. Husnot in *Melierax gabar* from Soudan where the authors registered two L., one in fusiform cells, other in round cells, quite different one from another.<sup>10</sup> Léger finds the L. of *Corvus corax* in a lymphocyte, the *L. ziemanni* of *Asio accipitrinus* in an erythroblast.<sup>11</sup>

If we turn now our attention to the study of the Schizogony of *Leucocytozoon* we see that the problem is far from being solved. One of the difficulties for such study is certainly that the infection is generally scanty in adult birds and only young animals give a chance for the finding of schizogonic stages. The description of Fantham<sup>12</sup> has not been confirmed by others. Moldovan<sup>13, 14</sup> describes young schizonts in lymphocytes or erythroblasts with 12-20 sometimes 30 nuclei (merozoites) in the organs in acute, very heavy infections.

I have not the pretension, neither is this an adequate occasion, to deal exhaustively with the literature on this subject. It will be done when better opportunities allow the writer to study this question more accurately and with more abundant and proper material. In any case, what has been stated will to a certain extent show how fascinating is the problem of *Leucocytozoa* and give perhaps to others, possessing a better material an excellent chance for the resolution of the problem.

The *Leucocytozoon* of our *Chloropsis* has not been studied in fresh blood but in films prepared just when the bird was dying. So the appearances seen cannot be attributed to some deformation due to making films some time after death as it has been pointed out by Wenyon.<sup>15</sup> The parasites belong to the round type. There is sexual dimorphism in the gametocytes, distinguishable not by the staining of the protoplasm, which seems the same in both cases, deep blue, but by the appearance of the nucleus: whereas on female gametocytes it is roundish or oval, weakly stained in



pale rose or not staining at all by Romanowsky (Leishman, May Grunwald-Giemsa), in male ones it is long, threadlike, irregular, staining in violet. The infection was very scanty and I must work in future with young birds, catching them on their nestle to see whether the advice given by França and confirmed by Léger will be of some profit in this case giving us a more abundant material. The shape of the parasites is round or oval. The protoplasm has many alveols and rarely some violet granules which have a chromatic tinge and are not at all of pigment nature. I cannot say anything on the nature of the infected cell whose appearances are figured in the plate. If generally the encircling mass is of nuclear nature, of such an irregular shape that I do not dare to make any hypothesis on its nature—specially because a study should be made at first on the normal Hæmatology of birds—there are rarely cases in which it shows an hemoglobinic (?) staining. I will only point the fact without any comment or hypothesis, which should be at present a mere speculation.

For this species which this note has only the intention to record I will give the name *Leucocytozoon chloropsidis*.

(c) A *Microfilarium* was present in large numbers in the blood and in the smears of organs. The nuclei are disposed in one compact column ending near the anterior pole by two bands. Anterior pole roundish, posterior one pointed. Three spots, the middle one in V. Cuticula with transverse striation. 90 to 120 microns long. 6 to 8 microns largest breadth.

#### Summary.

The following parasites of Indian birds found in Gôa are described or recorded:—

(1) *Herodias intermedias* Wagler: a *Giardia* abundant also in the intestine and which will be described later on; *Plasmodium herodiadis* n. sp.; *Hæmoproteus herodiadis* n. sp?; a *Microfilaria*.

(2) *Gallinula chloropus* L. *Plasmodium gallinulæ* n.sp.

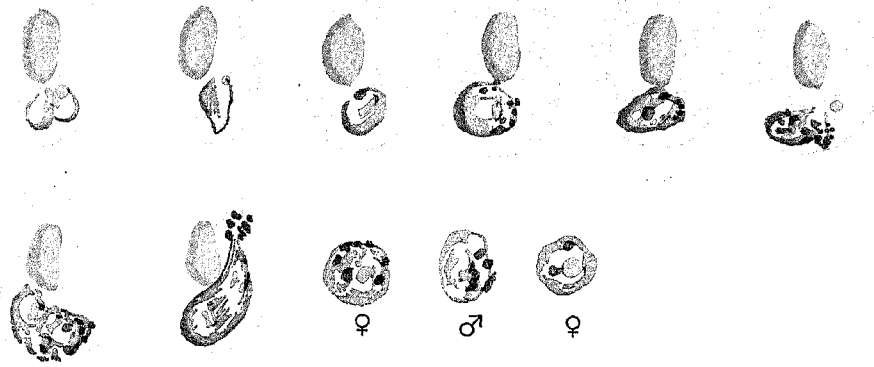
(3) *Machlolophus xanthogenys* (Vigors): *Hæmoproteus machlolopi* (Plimmer, 1912).

(4) *Chloropsis aurifrons davidsoni* Baker: *Plasmodium chloropsidis* (Scott, 1925); *Leucocytozoon chloropsidis* n.sp.; a *Microfilaria*.

#### BIBLIOGRAPHY.

1. Doflein and Reichenow .. *Lehrbuch des Protozoenkunde*, 1927.
2. Hartmann, E. .. "Three species of bird malaria: *Plasmodium precox*, *P. cathemerium* n. sp. and *P. inconstans* n. sp.," *Arkiv. für Protistenkunde*, 1927, Bd. 6.

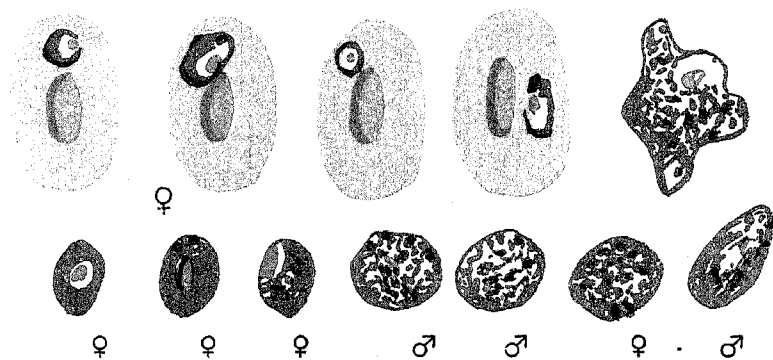
3. Russel, Paul F. .. .. "Avian malaria studies V. *Plasmodium capistrani* sp. nov. an avian malaria parasite in the Philippines," *Philip. Journ. of Sci.*, 1932, **48**, 269.
4. Wenyon .. .. *Protozoology*, Vol. II, 1364-87.
5. Danilewsky, V. .. .. "Developpement des parasites malariques dans les leucocytes des oiseaux (*Leucocytozoaires*)," *Ann. de l'Inst. Pasteur*, 1890, **4**, 426.
6. Léger, M. .. .. Discussion on the presentation of the paper of França C., "Leucocytozoon du geai, de l'épervier et de la bécasse," *Bull. Soc. Path. Exot.*, 1912, **5**, 17.
7. Mathis, C. et Léger, M. .. .. "Nature des cellule hôtes des Leucocytozoon," *Bull. Soc. Path. Exot.*, 1912, **5**, 77.
8. França, C. .. .. "Contribution à l'étude du Leucocytozoon des oiseaux du Portugal," *Bull. Soc. Path. Exot.*, 1912, **5**, 82.
9. Léger, M. .. .. "Hématozoaires des oiseaux de Corse," *Bull. Soc. Path. Exot.*, 1913, **6**, 515.
10. Léger André et Husnot, P. .. .. "Quelques hématozoaires d'un rapace diurne (*Melierax gabar*)," *Bull. Soc. Path. Exot.*, 1912, **5**, 74.
11. Léger, M. .. .. "Observations sur quelques Leucocytozoa d'oiseaux de la région de Reims," *Bull. Soc. Path. Exot.*, 1917, **10**, 28.
12. Fantham, H. B. .. .. "On the occurrence of schizogony in an avian *Leucocytozoon* (*L. lovati*) parasitic in the red grouse *Lagopus scoticus*," *An. of Trop. Med. de Parasit.*, 1910, **4**, 255.
13. Moldovan, J. .. .. "Sur le developpement du Leucocytozoon Ziemanni (Laveran)," *Bull. Soc. Path. Exot.*, 1913, **5**, 428.
14. Moldovan, J. .. .. "Untersuchung über den Zeugungskreis der Leucocytozoon Ziemanni (Lav.)," *Arch. für Protist.*, 1914, **34**, 249.
15. Wenyon, C. M. .. .. "Report of Travelling Pathologist and Protozoologist." Third Report—Wellcome Research Laboratories at the Gordon Memorial College, K hartoum.



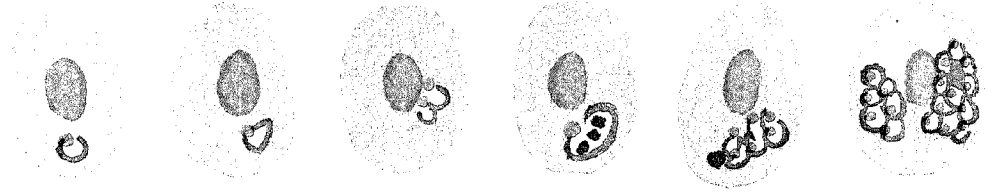
Plasmodium of *Herodias intermedia* Wagler.



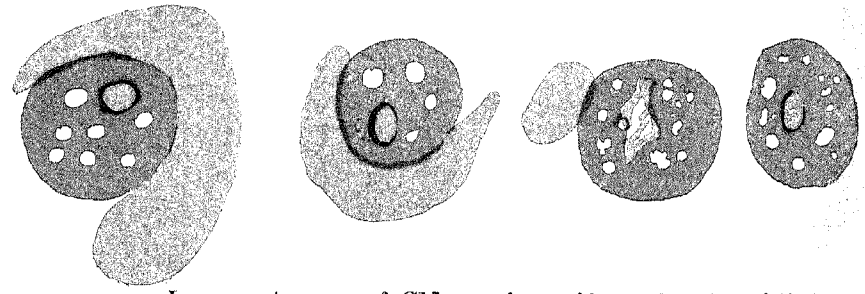
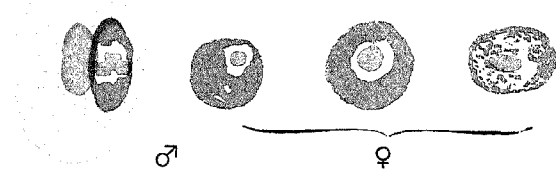
Hæmoproteus of *Herodias intermedia* Wagler.



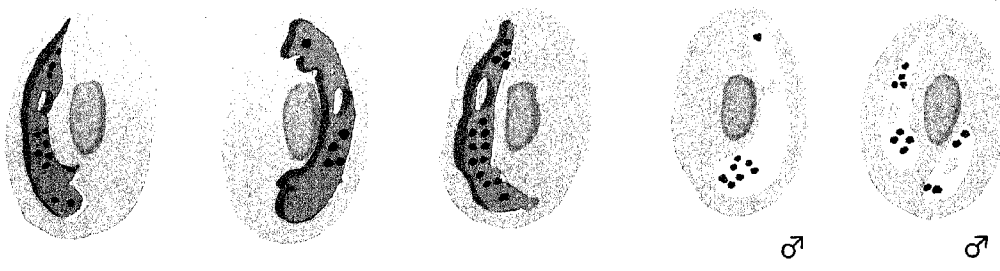
Plasmodium of *Gallinula chloropus* Linn.



Plasmodium of *Chloropsis aurifrons davidsoni* Baker.



Leucocytozoon of *Chloropsis aurifrons davidsoni* Baker.



Haemoproteus of *Machlolophus xanthogenys* (Vigors).