BLOOD PARASITES OF CORACIAS B. BENGHALENSIS WITH SPECIAL REMARKS ON ITS TWO TYPES OF LEUCOCYTOZOON.

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Among the birds of the genus Coracias we find in Wernyn: Coracias indica in whose blood Plimmer in 1912 and 1914 recorded a Haemoproteus. Coracias abyssinicus in whose blood Carpano found a Haemoproteus in 1913 in Eritrea, A. et M. Léger in 1914 a Haemoproteus in 1925 (specimen from Abyssinia); A. et M. Léger a Leucocytozoon in 1914 in Senegal. Coracias garrulus in whose blood Danilewsky recorded Trypanosoma avium in 1885 in South Russia; Cardamatis a Haemoproteus in 1909 in Greece; Wülker a Haemoproteus in 1919 in Macedonia; Danilewsky a Haemoproteus in 1889 in South Russia; Wülker a Leucocytozoon in Macedonia in 1919.

Our bird (one specimen shot at Corlim, department of Ilhas, identified by Dr. Baini Prasad) shows the following parasites:

(1) Haemoproteus.—Sexual dimorphism shown by the tinctorial reactions of the protoplasm which stains blue in female gametocytes and is colourless or slightly yellowish blue on males. The young forms of female gametes are small and more or less ovoid. When grown up, beautiful halterides embrace the nucleus of the red cells, which is displaced at the periphery. The protoplasm of the female is alveolar and does not stain uniformly; the blue tone is more pronounced on the poles and is lighter on the centre. Some specimens show the violet rings which we have found also in some other species of this genus. The halteride forms may be pointed with a kind of tail-like appendage, but when fully grown up they are broad, regular, lodging in their concavity the nucleus of the host cell. The pigment is yellow brown collected in minute granules or big dots and showing a tendency to collect on the poles. The nucleus of the female gametocytes is either compact or more or less granular. When free, the female gametocytes are roundish or oval, blue protoplasm, nucleus compact or irregular, pigment irregularly scattered over the body in clusters or isolated granules.
The male gametocytes show the same forms as the female ones, a little more irregular in young stages. The pigment shows the same appearance, perhaps more definitely collected on poles than in females in fully grown-up specimens. Nucleus under the form of an irregular spireme. When free, the male gametocytes are roundish, the protoplasm having a slightly violet bluish tone.

The red cell is slightly hypertrophied.

So many Haemoproteus have been recorded in other Coracias that it is very difficult to autonomous our species without consulting the original papers of the authors who studied them. The way in which the female gametocyte stains, with the poles deep blue and the centre in lighter tone constitutes for us one characteristic of this parasite as well as the colour and the form of distribution of the pigment.

We note however that none of the authors who studied the Haemoproteus of Coracias have named them: so rejecting the idea that it should be identified with H. danilewsky, as many authors have done concerning the Haemoproteids of birds in general, we are in a position to classify the species as Haemoproteus coraciae, which will take a trinominal designation, if not entirely similar to those already described. So we present this sp. as Haemoproteus coraciae benghalensis sp. n. (var. nov?).

20 microns

(2) Leucocytozoa.—Two species of Leucocytozoon have been found by us in this bird, distinct enough to be considered as separate species. Before describing them and making some remarks on a number of points which the study of these parasites suggests, we will refer to the literature already quoted in the description of our Leucocytozoon chloropsidis, and point out some other facts from other papers at our disposal.

Coles states that he has found Leucocytozoon in many birds, as jay, thrush, blackbird, pigeon, starling, moorhen and many others apparently all of the round type. He has never seen the spindle-shaped L. It may be argued that the forms I have met with have been the spindle-shaped specimens which have become rounded off after the blood was taken from the body. I am convinced that this has not been the case (except perhaps in a few cases in which the bird had been dead some time) as films were made and fixed at once by osmic acid vapour, or a fresh drop of blood was covered with a cover-glass
and examined immediately and in no case has there been any approach to the appearance of a spindle-shaped body."

As far as concerns this point of a transformation of the so-called spindle-shaped L. into round L., we have in this bird definite evidence that these two forms belong to two different species for the following reasons:

(a) the structure of the spindle-shaped L. is finely alveolar and it takes a more or less light blue tone to Leishmann or a light grey to Heidenhain's iron hæmatoxylin; that of the round L. is very compact, uniformly deep blue (Leishmann stain), or very dark (hæmatoxylin) with some white circular spots giving the impression of vacuoles devoid of any substance;

(b) the nucleus of the spindle-shaped L., central or sub-central, is long, oval or reniform, whilst that of round L. is circular, with a stronger nuclear membrane, and much smaller comparatively to the former.

(c) the forms of the spindle-shaped L. which become free never take the round, almost circular form of the second L. They are oval and show a structure entirely similar to the L. contained in those cellules à cornes of French authors.

"It is difficult to dogmatise as to the nature of the host cell; generally it seems to be a leucocyte or an immature red cell" continues Coles. We prefer for the present not to advance any hypothesis on the nature of the cells. But we possess enough elements to state that contrary to the opinion of the authors who say that each of these L. attacks one special cell (see the literature quoted on the description of L. chloropsidis), both these species of L. attack the same kind of cells. Their nucleus has the same structure in both cases and moreover, between the forms contained in the cellules à cornes and the free forms of the same parasite we find all the transitional stages where sometimes the poles of the cells are missing (one or both), or so slightly stained, almost vanished, that it is difficult to detect their existence. Among these transitional forms we find somewhere the nucleus alone remains attached to the L. just as in the case of the second L. On these grounds we are convinced that both these species of L. attack the same cell and that the second one has the property of lysing the cell protoplasm much more rapidly and completely than the spindle-shaped L., which however does it too, to a certain extent.

From the description and microphotographs illustrating the paper it seems that Coles has been happy enough to observe the divisional forms of the L. of the thrush.
In 1915 Laveran and Marullaz\(^*\) described as *Hemameba liothricis* (in Wenyon's book *Leucocytozoon liothricis*) a L. of the round type which for the convenience of description we will name *Type B*. There is nothing peculiar in this L. but the remarks of the authors concerning the nomenclature should be recorded: "Danilewsky a employé en 1889, le mot *Leucocytozoa* pour désigner d'une façon générale les parasites ayant leur siège dans les leucocytes, sans créer sous ce titre un genre bien défini. Depuis lors, on a appliqué la dénomination de *Leucocytozoon* à des Protozoaires qui appartiennent à des genres différents : Hémacibles des oiseaux et Hémogregarines des Mammifères parasitant des leucocytes et on a attribué à des genres différents des hématozoaires de même genre, suivant qu'ils parasitaient des hématies ou des leucocytes (Hémogregarines des mammifères) ; il est démontré que certains hématozoaires des oiseaux classée comme L. parasitent des hématies et que chez certains animaux à sang froid, la même hémogregarine parasite tantôt les hématies et tantôt les leucocytes. La confusion est donc complète."

The authors insist that this parasite lives in the red cells and add: "la conclusion à tirer de ces faits est que les hématozoaires endocellulaires ne doivent pas être classés suivant les cellules qu'ils parasitent, mais d'après leurs caractères morphologiques et évolutifs et que le genre *Leucocytozoon dont la création a été attribuée à tort à Danilewsky* (italic ours), ce nous semble, n'a pas sa raison d'être."

Commes\(^*\) describes a spindle-shaped L. in *Astur badius* var *sphenurus*, which he names *L. Martyi*.

Moldovan\(^*\) describes in the smears of organs "à coté des gamètes typiques, des formes incluses dans des cellules mononucléaires (lymphocytes ou érythroblastes) (?) qui d'après leurs caractères devaient être interprétées comme des schizontes jeunes du *Leucocytozoon Ziemanni*.... Les formes les plus jeunes entrent déjà en relation avec le noyau de la cellule parasitée, soit en s'accollant à lui, soit en pénétrant partiellement à son intérieur ou en l'entourant sans le déformer (italic ours). À un stade de développement plus avancé d'un certain nombre de ces trois variétés de schizontes (italic ours), on trouve le noyau du parasite en division. Les divisions nucléaires se succèdent rapidement et on arrive à trouver des schizontes possédant, comme je viens de dire, plus de 30 noyaux. Nous n'avons pas encore constaté la formation de mérozoïtes."

Wenyon\(^*\) remarks that this process is similar to that described by Coles and by Knuth and Magdeburg in *L. anseris* in Germany as occurring in the internal organs, either within mononuclear cells or free in the plasma and
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that the forms observed by these authors have been probably correctly interpreted as schizonts.

Marcel Léger describes: L. gentili from Fringilla petronia: “ce L. se trouve inclus dans des cellules hôtes arrondies. C’est un parasite des leucocytes mononucléaires.” And this classification of the host cells as well as the description of their form as arrondies is made, despite that “de la cellule hôte on ne perçoit, dans la majorité des cas, que le noyau rejeté à la périphérie, déformé, bosselé, allongé et bordant le parasite sur un tiers ou même une moitié de sa périphérie.” With such elements we do not know how it was possible to describe the host cells as roundish and classify them as mononucleares as the author does again when referring to the male gametocyte. He says: “le noyau est le seul vestige de la cellule hôte, est bien plus déchiqueté et se colore moins bien que celui d’un mononucléaire (italic ours) hébergeant une forme femelle.”

Describing le L. marchouxi from Turtur auritus there are at least some elements to try to classify the host cell. “la cellule hôte, arrondie, reste presque toujours intacte. Le protoplasma coloré en gris bleu (italic ours) par le Giemsa apparaît en bordure du parasite. Le noyau aplati et refoulé, se colore normalement ; il est certainement atrophié mais non en Karyolyse.”

Gris bleu is also the colour which takes the spindle-shaped poles of the host cell harbouring the L. of the A type. Will not then the host cells in both cases be of the same nature, differently deformed by the specific action of these types and will it not be more advisable to classify these types of L. as different genera?

We will now describe our Leucocytozoa, found either on the blood, or in the smears of the lungs, more abundant in these smears.

Type A.—Ovoid, included in a fusiform cell, whose poles are greyish blue to Romanowsky and slightly stained at Heidenhain’s iron hæmatoxylin. The nucleus of the host cell is attached to one side of the parasite which on this account has sometimes a bean-shaped form. But its real form is ovoid as we can see either in free forms or in those where the nucleus of the host cell occupies a central position vis-à-vis of the parasite. Many forms have been seen where the host cell has lost one or both poles, reduced only to the nucleus.

Protoplasm constituted by a fine alveolar net well marked with iron hæmatoxylin. At Romanowsky it stains blue, but lighter than in Type B. Sometimes large vacuoles are also seen, but the structure of the protoplasm, framed on this general type shows so many individual variations that it is impossible through it to try to find out sexual dimorphism.
We have found in one specimen only, among hundreds studied, a lot of very small rose granulations around the nucleus.

Sexual dimorphism is distinguished by the structure of the nucleus, recognisable not to Romanowsky but to iron hæmatoxylin. Its form is ovoid or bean-shaped, its position central or sub-central. There are two types of nuclei: one small with karyosome and centriole or only with centriole—it belongs to the female gametocyte; the other, much larger, often oval, elongated, or even bean-shaped, filled with granules not strongly siderophyl, and without any trace of centriole—it belongs to the male gametocyte.

The proportion of this *Leucocytozoon* to the other is from 35 to 5. We have met only adult gametocytes. No young form, no schizogonic stage was seen. Ratio of breadth to length 2/5 in intracellular forms and 4/7 in free forms.

*Type B.*—Spherical, attached to the nuclear substance of the host cell from which no other structure is seen. The constitution of this host cell nucleus seems entirely similar to that which lodges the Type A.

Protoplasm compact, deep blue to Romanowsky, much darker than that of Type A to iron hæmatoxylin. No alveolar net has been evidently seen, but many vacuoles, generally small having sometimes larger dimensions almost white and not stainable at all appear in the protoplasm. As all specimens seen had the same tone of coloration and the nucleus with karyosome, it seems to us that we have found only one type of gametocytes. Forms of different sizes, the free ones absolutely spherical and not ovoid as in Type A.

The study of these Leucocytozoa shows that we are definitely dealing with two species autonomous and independent and it convinces us that while the first type may be classified as *Leucocytozoon* (genotype universally accepted: *L. ziemannii*), the second one has, as all the parasites of this type, enough characters to constitute an independent genus to which the name of Marcel Léger should be attached. For the present we do not advance more on this line, hoping for further material to settle this point.
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For classifying these parasites we are not aware of the characters of the L. of Coracias abyssinicus and of Coracias garrulus. As no specific name is registered in Wenyon, always so careful to record all specific names, it seems that we are allowed to give the following nomenclature:

Type A—Leucocytozoon coracici benghalensis.
Type B—Leucocytozoon (or new genus to be created?) sp.

A microfilarium was present in such a scanty number, that we have found only four specimens in all the slides examined. Provided with a sheet. Anterior pole rounded. Posterior pole tail like, free on its last part from the nuclear mass which begins at six to seven microns from the anterior pole, as a compact mass. Three spots, the median (situated sometimes a little behind) in V or crescent form. In the middle of the body there is a very regular ovoid body, stained in rose by Leishmann and corresponding to the central Viscus of Manson. The caudal spot oval, very regular. Length 50-55. Breadth 4—5 microns.

N.B.—One specimen of Coracias benghalensis shot at Diu (identified by Mr. S. H. Prater from Bombay) did not show any parasite at all.

BIBLIOGRAPHY.

1. Wenyon's Protozoology, 2, 1370.
Coracias b. benghalensis L.