

NOTES ON THE PARASITES OF MOSQUITOES  
FOUND IN INDIA BETWEEN 1895 AND 1899.

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ON reading the excellent paper on the parasites of the Culicidae recently published by Dr Léon Dyé<sup>1</sup>, I was struck by the fact that several of the organisms mentioned by him had been seen and described by me long ago in the course of my original studies on malaria and mosquitoes carried out in India during 1895–1899. My observations were, however, recorded for the most part in Indian medical publications, which are little accessible to European readers, and have been so far forgotten that some of the parasites of mosquitoes seen by me have been recently rediscovered by various observers. It may therefore be of interest—perhaps not alone from the historical point of view—to resuscitate these old records and compare them with more recent and exact work. I may add that attempts were made by me to infect men with two of the organisms which I observed. My writings on the subject were contained in the following publications, which are here numbered for reference :—

I. Ross, R. (XII. 1895.) *Observations on the metamorphosis of the malarial parasite within the mosquito. Transactions of the South Indian Branch of the British Medical Association*, Vol. VI. No. 5. Lawrence Asylum Press, Madras, India.

In this publication on page 346 a gregarine of mosquitoes is described, with rough drawings (badly reproduced in printing). Letters from Manson confirming my observations and discussing the possibility of this gregarine having a connection with the parasites of malaria are added. The article is dated the 11th October, 1895.

<sup>1</sup> *Archives de Parasitologie*, vol. IX. pp. 5–77. 1905.

II. Ross, R. (XII. 1896.) *Some experiments in the production of malarial fever by means of the mosquito.* *Ibid.*, Vol. VII. No. 3.

In this second paper I described twenty-six attempts to infect men by means of water in which the larvae of mosquitoes had been kept, and also by bites of mosquitoes. Of these experiments some were made (on myself among others) with the sporocysts of the gregarine described in my first publication (p. 98 *et seq.*). The result of the experiments was declared to be indefinite (p. 104); but three rather curious partial successes were obtained, the remaining experiments being wholly negative. This paper was read on the 30th October, 1896.

III. Ross, R. (II. 1898.) *Report on a preliminary investigation into malaria in the Sigur Ghat, Ootacamund.* *Ibid.*, Vol. VIII. No. 5.

On page 147 of my third paper there is given a list of parasites found by me in mosquitoes. These parasites were (1) *Filaria sanguinis hominis* (*F. bancrofti*); (2) a nematode; (3) a fungus; (4) *Gregarina culicis*; (5) spores of a protozoal parasite; (6) certain coccids; (7) "sporangia," giving rise to flagellulae and amoebulae; and two objects of doubtfully parasitic nature. Of these, Nos. 4, 5, 6, and 7 are briefly described. The paper, from which the date was omitted by the editor, describes researches carried out from April to June, 1897, as stated on page 145.

IV. MANSON, P. (21, III. 1896.) Goulstonian Lecture, No. 2. *British Med. Journ.*, No. 1838.

On page 717, Manson confirms my description of the *Gregarina culicis*.

V. Ross, R. (26, II. 1898.) *Pigmented cells in mosquitoes.* *British Med. Journ.* No. 1939.

On page 550 I describe finding parasites No. 7 of publication III in a "grey" mosquito (*C. fatigans*) which contained also the zygotes of a *Plasmodium*.

Of the foregoing publications Nos. II and III were reprinted in the *Indian Medical Gazette* for 1897 and 1898, and No. I in the *Indian Lancet*, 1896.

I will now make some notes on the parasites mentioned in publication III, and will take the opportunity to add a few facts regarding them which came to my knowledge after I wrote the papers referred to above.

Parasite (1). *Filaria bancrofti* needs no comment.

Parasite (2), a nematode: three large, active, but immature nematodes were found in the stomach cavity of a single larva of a *Culex* (probably *C. fatigans*) obtained near Ootacamund, India. I never saw these parasites again. It is possible that they belonged to the genus *Agamomermis* Styles.

Parasite (3) may have been only a commensal intestinal fungus.

Parasite (4), *Gregarina culicis*. This was described at some length in my publications I, II, and III, confirmed by Manson in IV. Comparing these papers we shall find that the youngest gregarines are described as being found in the perivisceral cells of the youngest larvae. Growing in size, they escape from the cell host, become active, and, when the larva develops into a pupa, migrate into the malpighian tubes. There they become encysted "with or without conjugation" (IV), and produce a large number of pseudonavicellae which are expelled with the faeces of the imago, either into water or upon the human skin (III). I well remember collecting these pseudonavicellae from a droplet of fluid ejected by a *Stegomyia* on my own hand. Contrary to expectation, I failed in infecting young larvae by means of the pseudonavicellae (III, page 150). The length of the first gregarine seen by me is given (I) as reaching to 200  $\mu$ ; but subsequently I found much smaller varieties, and indeed conjectured that there might be several species (III). The organism was named *G. culicis*. In II several attempts to infect men by means of the pseudonavicellae are recorded. One of the cases suffered from fever five days after the pseudonavicellae were administered, two doubtful plasmodia being found in his blood. This result was probably a coincidence. I myself swallowed about 3000 pseudonavicellae on the 5th and on the 21st November, 1895, without the smallest reaction. These gregarines were frequently found by me subsequently in various parts of India, but only in my "brindled" mosquito, that is in *Stegomyia*. There is no doubt that the smaller varieties observed by me were of the same species or genus as the gregarine of *Stegomyia fuscata* recently so well described by Marchoux, Salimbeni, and Simond<sup>1</sup>. I may add that subsequently the mature gregarines were frequently seen by me in the coelom as well as in the intestinal cavity; and that I have constantly described this parasite in my lectures given since 1899. Dyé has noted my work in this connection in his paper. It is interesting to remark that Marchoux, Salimbeni, and Simond suspected a relation between the gregarine and yellow fever, just as I had suspected a similar relation between it and

<sup>1</sup> 1903. *Ann. de l'Inst. Pasteur*, vol. xvii. p. 713.

malaria. I am, however, not yet absolutely convinced that it has no relation with man, more especially because, unlike these authors, I am not sure that the pseudonavicellae can directly infect the young larvae.

Parasite (5) of publication III, the "spores of a protozoal parasite," consisted of a collection of exactly eight spores closely packed within an oval envelope, which was probably the shell of the cell which contained the parent cytozoon. Each spore was oval, measuring  $2\mu$  by  $5\mu$ , refractive, apparently hard, and containing a circular vacuole at one focus of the ellipse. Numbers of these little clusters of spores were found within the sheath of the ventral nerve trunk of the imago, especially in a small species of *Stegomyia* occurring near Ootacamund, and also, so far as I remember, in *C. fatigans* of Ootacamund itself. The mature spores appeared to escape in an unknown manner from the imago, and could be found in the water at the bottom of the vessel in which the insects were kept. There they seemed to grow much in size; and on several occasions I thought I observed amoebic embryos escaping from them. Since that time I have never seen these bodies again. They were not the same, I think, as the Myxosporidia with eight sporozoites mentioned by Grassi and also, apparently, by Christophers and Stephens.

Parasite (6) of publication III was a well-defined oval organism,  $8\mu$  by  $4\mu$  in size, lying by the side of the nucleus within the stomach cells of a few individuals of *Culex sp.* Each possessed a vacuole (?) surrounded by a faint granulation. They probably belonged to the Coccidiidae; but were never seen again by me.

Parasite (7) of publication III may prove to be of importance in connection with Schaudinn's work on the development of the Haemosporida of *Athene noctua* in *Culex pipiens*, and I therefore think it advisable to describe my past observations of them at some length. They were frequently found by me after the date of publication III, generally in *Culex fatigans*, and once in an Anophelina; they occurred in larvae as well as in adults; a person to whom they were given by the mouth acquired fever; and I still possess a preparation containing them, made in Calcutta in 1899 from a mosquito containing *Proteosoma*. It is probably these parasites which have been rediscovered by Chatterjee, Stephens, and Christophers and Léger in Anophelines, and which have been named by the last observer *Chrithidia fasciculata*.

In publication III, page 148, I described them briefly as follows. "(7) Sporangia in the stomach of some newly-hatched adult mosquitoes from the Wesbury wells. Under water the sporangia give rise at once

to thousands of *flagellulæ* and also (?) *amoebulæ*. The *flagellulæ* are about  $8\mu$  by  $2\mu$  in size with numerous minute black points in their substance and have a single long flagellum. They grow slightly in size and distinctness and attack the tissues of the dead host. I am inclined to think that naturally they descend to the intestines, where they adhere and undergo further unknown development, but they may be passed out upon the human skin during haustellation. The stomachs containing these *sporangia* were given to a volunteer, who was attacked with slight fever on the 5th day; but I found no parasites in his blood. Owing to the paucity of specimens I was not able to study the original *sporangia*, but they seemed to be without an inclosing cyst wall and to lie unattached among the food residues in the stomach of the young imago; in short they consisted of free clusters of *flagellulæ* closely adhering at first but separating under water. At one time I thought that the *flagellulæ* might be derived from the sporocysts of *Euglena* which abounded in the well and were swallowed in large numbers by the larvae."

Although this description is not very lucid, it will suffice, when considered together with the specimen now in my possession, to enable us to recognise the similarity of the parasites to those described by Chatterjee and Léger<sup>1</sup>. By "*sporangia*" "which give rise at once to thousands of *flagellulæ* and *amoebulæ*," I meant "*colonies radiées*," that is, radiating clusters, of small active bodies, which were either amoebae or possessed a single long flagellum, and which subsequently freed themselves and moved independently in the intestine of the host. I studied them frequently afterwards and now collect my observations in the following note.

The parasites were found by me in India in various species of *Culex*, principally *C. fatigans*, at Ootacamund, at Kherwara in Rajputana, at Calcutta, at the foot of the Himalayas near Darjeeling, and in Assam. They occurred in the larva, pupa, and imago. Sometimes they existed in a considerable proportion of the insects examined, especially at Ootacamund (2,500 metres above sea-level), where they abounded in a species of *Culex* which I remember was different from *C. fatigans* though very like it. I never met with them in *Stegomyia*, but certainly saw them on one occasion either in Sierra Leone or at Ismailia—I forget which—in an Anopheline. Being immersed in a study of the *Plasmodia*, I had no leisure to examine the *Chritidia* very minutely; but they presented roughly the same appearance in all the *Culicidae* in which I observed them.

<sup>1</sup> See the paper by Dyé, *Arch. de Parasitologie*, 1905, No. 1.

After writing publication III, I found them in several large *larvae*. When the larva was first dissected, they existed in the stage of "colonies radiées" among the contents of the middle intestine, which consisted largely of *Euglenae* and their cysts. The "colonies radiées" were at first immotile; but when the water in which the larva was dissected (I did not use salt solution) reached the contents of the stomach, the individual organisms of which the colony was composed began to show very active movement and to break away from the parent mass. They were then seen to consist either of small amoeboid bodies or of elongated organisms possessing a single long flagellum. The latter were very motile and moved easily in all directions, often attempting to thrust their flagellum into the cells of the larva—at least, so it seemed to me. Both the amoeboid and the flagellate forms possessed several vacuoles, and also numerous minute granules which appeared almost black when the field of the microscope was darkened. In some of the flagellate forms I thought I detected the rudiments of an undulating membrane; and on several occasions I fancied that the amoeboid forms began to develop flagella. They lived for a long time and perished only when the preparation became dry. As the flagellate forms resembled very young and small *Euglenae* without chlorophyll, I thought at one time that they might even be derived from these organisms, which are often swallowed by the larvae (III).

In the pupae of *Culex sp.*, of which I examined a considerable number at Ootacamund, precisely the same forms were to be seen—namely "colonies radiées" in the stomach, quickly breaking up in the presence of water into *amoebulae* and *flagellulae*.

The same forms also occurred in the imagines when newly emerged from the pupa; but here I noticed that from the first many free *amoebulae* and *flagellulae* already existed in the stomach together with the "colonies radiées." In imagines which were a little older, only the free forms occurred as a rule, and, moreover, only the flagellate forms were found—thus tending to confirm my impression that the amoeboid forms ultimately became flagellate. I now observed also that these *flagellulae* had often passed from the middle intestine into the lower intestine. Here they were generally attached, by means of their long flagellum, to the epithelial cells lining the cavity, and sometimes occurred in such large numbers that their bodies were contiguous, and gave the appearance of a second layer of cells attached to the epithelial layer of the intestine. Shortly after the death of the host however, and, as before, on the addition of water, most of the

parasites rapidly detached themselves and moved freely in the medium. At this stage they were all flagellate. I was unable to trace their life-history beyond this point, and cannot say how they succeed in passing from the adult back again to the larva. I thought, however, that they might be evacuated during haustellation upon the human skin, and indeed suspected at that time that they might possibly constitute the mosquito-stage of the *Plasmodia*. In order to test this hypothesis I made the experiment mentioned in III. I gave the stomachs of several mosquitoes containing numerous *Chrithidia* to a native of India, who assured me that he had not suffered from fever for a long time. Five days later he came to me with fever—a singular but certain fact; but I could find no *Plasmodia* in his blood, even after careful search. This was in June, 1897, at Ootacamund; and unfortunately I was obliged to leave that town shortly afterwards, so that I never saw the patient again.

Subsequently I came to think that these apparently positive results were produced in some manner by the subjects themselves (all natives of India) in order to obtain a higher reward from me. Of this I cannot speak with certainty; but the facts were exactly as recorded by me in II and III.

A few months after these observations (August, 1897), I succeeded in finding for the first time the zygotes of *Plasmodium falciparum* Welch in Anophelines at Secunderabad, and also those of some other *Plasmodium* in a single *Culex fatigans*. This mosquito contained also the *Chrithidia* (IV).

Subsequently they were observed from time to time in various *Culex* sp., and I remember seeing them on two occasions moving rapidly in the midst of freshly ingested blood, just as Chatterjee has described, when they much resembled trypanosomes. But there was little possibility of forming a wrong conclusion, as many of them could at the same time be seen adhering to the lower intestine, where they could scarcely have arrived so early if they had been swallowed with the blood. In fact it was evident that they had been already present in the insects before these were fed on the blood. As I have mentioned, I once found them certainly in an Anopheline. Curiously enough, however, I observed them only on a single occasion among the large numbers of *Culex fatigans* in which I succeeded in cultivating *Plasmodium* (*Proteosoma*) *relicta* in 1898 and 1899, and that was in the insect of which I still possess a preparation (made in Calcutta and dated 21st Feb. 1899). Many of the birds on which these insects had been fed contained *Halteridium* as well as *Proteosoma*; if therefore the

*Chrithidia* are developed in any way from these *Haemosporidia* it is strange that they should not have occurred more frequently in these numerous experiments.

The *Chrithidia* were never seen by me outside the intestine of the hosts, that is, in the tissues; but this may have been due to the fact that my studies of them were not exhaustive. Many of the more rounded forms, which I called the *amoebulae*, were truncated, just as described by Léger. I now think that these *amoebulae* were probably merely transitional dividing forms.

I made no attempt to stain the organisms; but from the researches of Léger it appears that they have a structure which recalls that of trypanosomes. What are almost certainly the same parasites have also been stained and figured by Stephens and Christophers; see page 122 of their book, "The Practical Study of Malaria," first edition, University Press, Liverpool, where a list of parasites seen by them is given, with a drawing of stained *Chrithidia* adhering to the hind-gut. They do not state in which species of mosquitoes they found the organisms.

On the whole I can find little reason for supposing that the *Chrithidia* are developed from *Haemosporidia*. They appear to be derived from organisms contained in the intestine of the larva and probably swallowed by it.

What relation do they bear to the trypanosoma-like bodies which Schaudinn found in a percentage of the *Culex pipiens* which he had fed on *Athene noctua* containing *Halteridium*? He conjectured that the trypanosoma-like bodies were "neutral" derivatives of the zygotes of this *Halteridium*. Might they not have been merely *Chrithidia* already present in the insects with which he worked? The *Chrithidia* exists in Anophelines and also in several Indian species of *Culex*, especially in *C. fatigans*, which may almost be called the *C. pipiens* of India. It is probable therefore that they exist also in the *C. pipiens* of Southern Europe. I can find no passage in which Schaudinn excludes this possibility. We must note that his trypanosoma-like bodies occurred only in a percentage of his mosquitoes, and that they were found adhering to the cells of the lower intestine—facts recalling the *Chrithidia*. Moreover the experiments of Novy and of Thiroux, and the general parasitological experiences of many of us, are scarcely in favour of the theory that the *Haemosporidia* are specifically connected with the trypanosomes and spirochaetes. I think that some of the facts mentioned above suggest that the enquiry into this matter should be renewed.