A CONTRIBUTION TO THE STUDY OF THE HAEMATOZOON PARASITES OF BATS. A NEW MAMMALIAN HAEMOPROTEID, *POLYCHROMOPHILUS DEANEI* N. SP.[1]

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(With 1 plate)

In 1938, Professor Leonidas Deane and Dr. Maria Deane discovered two bats (*Glossophaga soricina soricina* PALLAS, 1766) infected with a malaria parasite. They were caught at Piratuba, município of Abaetetuba, Pará State, in a banana plantation in the forest. Camera-lucida drawings were made of the parasites in the blood, but the specimens were lost and the description was only published 13 years later (DEANE & DEANE, 1961). Before and after 1938, blood films of bats had been examined for parasites, but no other example of a malaria parasite had been reported from South America. There is only one other record of such parasites in the New World — the finding by WOOD (1952) of "malaria parasites" in some specimens of *Antrozous pallidus* in California and *Pipistrellus hesperus* in Texas — unfortunately, their description is meagre and unillustrated.

Haemosporidian parasites of any sort are rare in New World mammals, and this occurrence in bats in the Americas presents an interesting problem in zoogeography. We felt that further investigations should be made in order to determine the true incidence and nature of the chiropteran parasites in Brazil. The Cambridge Medical Students' expedition (under Pereira) in 1969 was asked to make a preliminary survey in Piratuba, but the results, like the Deanes' later investigations, were negative.

In October 1969, we were provided with a small cruiser by Petrobras in order to explore the territory and the nearby river systems. Our intention was to concentrate on the collection of specimens of *Glossophaga* (the "beijaflor" or "flower kisser") in the vicinity of the Deanes' type locality. The environment of the actual place, Piratuba, had entirely

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changed in the last twenty years and little forest or banana plantations remained. We accordingly selected three other sites for observations: (1) on the banks of the Rio Abaete, about 10 kms from its mouth, (2) a similar distance up the Rio Uruaenga between Abaetetuba and Barcarena and (3), on the Rio Maracapuçu towards the Foz do Tocantins. These places were relatively untouched and consisted of small "sitios" cut out of low vegetation ("capoeira") on the edge of the river, with a few bananas, coffee bushes, palms and other fruit trees.

Mist nets were erected in these places from ground-level to a height of about 7 ft. They were set up in the late afternoon and bats were caught at intervals up to nearly midnight. The bats were then put separately into canvas bags and taken back to the cruiser which was anchored lower down the tributary; the actual site had to be reached by dinghy as the water became too shallow for the larger vessel. The following morning the catch was roughly identified and those bats resembling *Glossophaga* were processed. Ectoparasites were collected and fixed in 10% formol saline; thick and thin films were made from the host blood; pieces of spleen, liver, kidney, heart and lung were fixed in 10% formol saline, and the bat was then preserved for identification.

RESULTS

Specimens of the long-tongued *Glossophaga* were rare and only three of the ten "possibles" were finally identified as belonging to this genus. We ignored the larger phyllostomatids etc., but took one small, short-tongued bat *(Myotis nigricans nigricans* SCHINZ, 1821) from the Maracapuçu sitio for luck. Ironically it was only in this specimen that the malaria parasite was found; it proved to be also the only bat on which nycteribiid flies were present in the fur and the only one which had an enlarged, black spleen.

The remaining nine bats were all caught on the banks of the Uruaenga and showed the following parasites in the blood:

Glossophaga soricina (Total, 3)
1 with *Trypanosoma (cruzi*-type)
2 with rickettsial-like bodies in erythrocytes

Choeroniscus minor PETERS, 1868 (Total, 3)
1 with *Trypanosoma* sp., (not *T. cruzi*-type)

Lonchophylla thomasi ALLAN, 1904 (Total, 3)
2 with *Trypanosoma (cruzi*-type)

The identity of these trypanosomes will be discussed more fully in a subsequent paper. The nature of the curious "dots" found in the erythrocytes of *G. soricina* is obscure. They are very small and occupy only a localised, usually central, position in the cell. In morphology they resemble *Cytoecetes* of leucocytes. We first thought that they were *Grahamella,* but they have neither the right shape nor the more

generalised distribution of that organism. They do not appear to be remnants of the erythrocyte nucleus, and it seems most likely that they are of a rickettsial nature.

Polychromophilus deanei sp. nov., the "malaria parasite" in the single specimen of *Myotis nigricans*, had such a striking morphology that it was immediately recognised as a species of *Polychromophilus*. The infection was scanty and was originally missed in the thick film where the cytoplasm had become unusually transparent. Only gametocytes were present in the blood — a feature of all genera of the Haemoproteidae, to which *Polychromophilus* belongs. The microgametocytes were less common than the macrogametocytes, but both had the characteristic oval snape and, when mature, appeared to be devoid of any erythrocytic covering.

The microgametocyte (figs. 1 & 2) is approximately 8 μ long and 6 μ wide. It stains a brick red colour and the nucleus occupies nearly half of the parasite. In the thick film, however, this diffuse nucleus rounds up into a compact red mass; in the thin films a small, dense body (karyosome, or possible centriole) is visible on the edge of the nucleus. The parasite's cytoplasm is tenuous and the dark brown or black pigment consists of irregularly scattered grains of different sizes.

The macrogametocyte (figs. 3 & 4) is about 7.5 — 8.0 μ long and 6.0 μ wide. The blue colour of the cytoplasm, following colouration with Giemsa stain, immediately differentiates it from the male. The pigment is much the same as in the male, except for its somewhat wider distribution. The round karyosome is either embedded in the nucleus or lies some distance apart.

Only two immature gametocytes were seen (fig. 5). At this stage, the parasite is clearly intracorpuscular and has a round outline. The parasite is small and the cytoplasm is apparently not amoeboid. A conspicuous nucleus lies at one side and small grains of pigment are already present.

Sections were prepared from the organs (spleen, liver, kidney and heart) of this bat and were stained by the Giemsa-Colophonium method (BRAY & GARNHAM, 1962) but a prolonged search revealed no exoerythrocytic schizonts.

Nycteribiid flies (*Basilia* sp.) were present on this bat; several escaped, but three were collected and fixed in formol-saline. After identification, the heads and thoraces were cut off and the abdomens were prepared for sectioning.

In two of the flies, oocysts were found adjacent to or in the vicinity of the midgut and measured about 60 μ in diameter (fig. 6). Each oocyst was enclosed by a conspicuous, amber-coloured wall, which appeared "crinkled" in some sections. The contents were approaching maturity and rows of sporozoites were clearly visible along folds of residual cytoplasm. Many hundreds of sporozoites were present; they appeared to be about 8 μ in length, though when free they are probably slightly

longer. The width of the sporozoite appeared to be rather wider than that of *Plasmodium.* In one oocyst, numerous dark brown bodies were present; they were about 3 μ in diameter and probably represented Ross' "black spores". These structures, together with the unusually conspicuous wall in some oocysts, probably indicated that degeneration was beginning to take place.

Only a small number of oocysts were present in each fly, and a prolonged search under high magnification failed to show any earlier stages in their development. The salivary glands were identified in the sections but no sporozoites were visible in the acini.

DISCUSSION

Instead of finding the Deanes' parasite again in *Glossophaga,* we discovered an allied or identical organism in the single specimen of *Myotis nigricans* which we collected near the original locality. In the Deanes coloured plate, immature parasites and macrogametocytes are illustrated. The authors remark on the ellipsoid shape frequently assumed by the parasites which reach a length of 5.0 — 7.0 μ. Only females are shown in their Plate, and these contain a localised nucleus and numerous dark pigment granules. Owing to the discovery of our parasite in a different host, and in the absence of the original material, it is difficult to be certain that the same species was present on both occasions. Nevertheless, the general morphological features are similar, and we are pleased to take this opportunity of acknowledging the help we have received from Professor Leonidas Deane and Dr. Maria Deane by naming the present organism (as defined below) *Polychromophilus deanei* sp. nov.

This is the third occasion on which sporogonic stages of *Polychromophilus* have been demonstrated. CORRADETTI (1936) found sporozoites in a nycteribiid fly *(Listropoda)* taken off a bat near Rome (Dionisi's type locality of the genus), but he made no observations on the blood of the animal. MER & GOLDBLUM (1947) incriminated nycteribiids *(Penicillidia* spp) as vectors of *Polychromophilus* sp. in *Myotis* spp. and the latter author also saw oocysts. The descriptions given by these workers are meagre, but they all noted the "stumpy" appearance of the sporozoites. The rate of infection of haemoproteids in ectoparasitic flies is usually high, as the insects have continuous opportunities of feeding on the host; further material from Pará will probably reveal details of the complete sporogonic cycle.

An interesting zoogeographical problem is presented by this biocenose, comprising *Myotis, Basilia* and *Polychromophilus,* all of which elements are now shown to extend to South America and thus attain a cosmopolitan distribution. The blood parasite is common in this genus of bat in Europe and the Middle East, and probably occurs also in Malaya, Far East, Australia and Africa (see GARNHAM, 1966). But how did the full complex reach the Americas? The vespertilionid bats are thought to have reached the New World across the Behring Straits

and to have gradually colonised and evolved as they spread southwards; certainly the primitive genus *Myotis* has been most successful and is the predominant bat in many places — no doubt its ectoparasites (including nycteribiids of the genus *Basilia)* inevitably accompanied the bat in its migrations. The genus *Basilia* is found both in the Old and the New World, and in the latter, according to THEODOR (1957), it is the chief ectoparasite of *Myotis.*

Presumably the whole complex of parasite and two hosts travelled together and has persisted since the earliest times. Other vespertilionid bats apparently shared in the infection, which was found in a few specimens of *Antrozous* and *Pipistrellus* by WOOD (1952) in California, though the exact nature of the parasite and the vector have not been determined *. The spread of the infection in closely related bats could easily take place owing both to the lack of host specificity of the nycteribiids, and to the habit of different types of insectivorous bats of sharing the same roosting places (WYNNE-EDWARDS, 1962).

The transportation of another haemoproteid parasite, *Haemoproteus columbae,* with its vectors *Pseudolynchia* spp. on the pigeon host is a parallel example in birds. This association is found in nearly all countries of the world, including Brazil, where ARAGÃO (1908) was the first worker to describe the complete life-cycle. The actual arrival in the Americas is of course unrecorded, and the infection itself may be recent, e.g. infected pigeons may have been blown across the Atlantic, or bats may have been transported in the holds of ships. The parasites may then have become established in the Americas like the virus of yellow fever, *Onchocerca volvulus* and, possibly, the agents responsible for malaria and plague.

The blood of bats in the New World has been extensively examined for *Trypanosoma cruzi* (e.g. in the recent summary by MARINKELLE, 1968), but with the two exceptions mentioned above, no malaria parasites have been observed. Further investigations, at least in Pará and California, may provide a better idea of the incidence of *Polychromophilus.*

DESCRIPTION

Polychromophilus deanei sp. nov.

Ovoid or subspherical gametocytes in erythrocytes; approximately 8 μ long and 6 μ wide; dark pigment granules of variable size; mature forms apparently devoid of erythrocytic envelope. Mature oocysts about 60 μ in diameter, sporozoites relatively short and thick.

VERTEBRATE HOST. *Myotis nigricans nigricans* (SCHINZ, 1821).

INVERTEBRATE HOST. *Basilia* sp.

* One of us has seen Dr. Woods' slides. The parasite appears morphologically distinct from that we describe here.

LOCALITY.	Low forest ("várzea") on bank of Rio Maracapuçu, Município of Abaetetuba, Pará, Brasil.
TYPES.	Wellcome Museum of Historical Science, London.

SUMMARY

Polychromophilus deanei n. sp. (Haemosporidiidea, Haemoproteidae) is described from a vespertilionid bat, *Myotis nigricans nigricans* (SCHINZ, 1821), captured on the Rio Maracapuçu, Município of Abaetetuba, Pará, Brasil.

Mature oocysts were found in 2 specimens of the ectoparasite *Basilia* sp., taken from the infected animal.

The parasite is probably the same as that previously described in another bat, *Glossophaga soricina soricina,* from the same general area, by DEANE & DEANE in 1961.

SUMÁRIO

É descrito o encontro de *Polychromophilus deanei* n. sp. (Haemosporidiidea, Haemoproteidae) em morcêgo *Myotis nigricans nigricans* (SCHINZ, 1821), capturado no rio Maracapuçu, Município de Abaetetuba, Pará, Brasil.

Oocistos maduros foram detectados em espécimens de ectoparasitas *Basilia* sp., retirados do animal infectado.

O parasita em questão constitui-se, provàvelmente, o mesmo prèviamente descrito por DEANE & DEANE em 1961, em outro gênero de morcêgo *(Glossophaga s. soricina)* procedente da mesma área em geral.

ACKNOWLEDGMENTS

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The coloured illustrations were kindly painted by Prof. J. V. SCORZA.

REFERENCES

ARAGÃO, H. de B. (1908). Über den Entwicklungsgang und die Übertragung von *Haemoproteus columbae*. *Arch. Protistenk, 12,* 154-67.

BRAY, R. S. & GARNHAM, P. C. C. (1962). The Giemsa-Colophonium method for staining protozoa in tissue sections. *Indian J. Malariol., 16,* 153-5.

CORRADETTI, A. (1936). Alcuni protozoi parassiti di *Nycteribiidae* del Genere *Listropoda*. *Ann. Igiene, 46,* 444-8.

DÈANE, L. M. & DEANE, M. P. (1961). Sôbre dois haemocitozoarios encontrados em mamíferos silvestres da região amazônica. *Rev. Inst. Med. Trop. S. Paulo, 3,* 107-10.

GARNHAM, P. C. C. (1966). "Malaria parasites and other Haemosporidia". Oxford, Blackwell's Scientific Publications.

GUIMARÃES, L. R. & D'ANDRETTA, M. A. V. (1956). Sinopse dos *Nycteribiidae* (Diptera) do Nôvo Mundo. *Arqu. Zool. Estado S. Paulo, 10,* 1-184.

MARINKELLE, C. J. (1966). Importancia de los Murcielagos del tropico Americano en la salud publica. *Antioquia Med., 16,* 179-194.

MER, G. G. & GOLDBLUM, N. (1947). A haemosporidian of bats. *Nature. Lond., 159,* 444.

THEODOR, O. (1957). Parasitic adaption and host parasite specificity in the pupiparous Diptera. First symp. on host parasite specificity among parasites of Diptera. 50-63. Neuchâtel, Attinger.

WOOD, S. R. (1952). Mammal blood parasite records from S. W. United States and Mexico. *J. Parasit., 38,* 85-6.

WYNNE-EDWARDS, V. C. (1962). Animal dispersion in relation to social behaviour. Edinburgh, Oliver & Boyd.

PLATE I

Figs. 1 & 2. Mature microgametocytes of *Polychromophilus deanei* n. sp. in the peripheral blood of the bat *Myotis nigricans nigricans*.

Figs. 3 & 4. Mature macrogametocytes, with an un-infected erythrocyte for comparative size.

Fig. 5. Young gametocyte.

Fig. 6. Mature oocyst, containing sporozoites, as seen in sections of the nycteribiid ectoparasite *Basilia* sp., taken from the infected bat.

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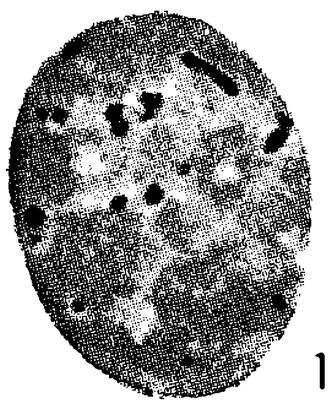
PLATE I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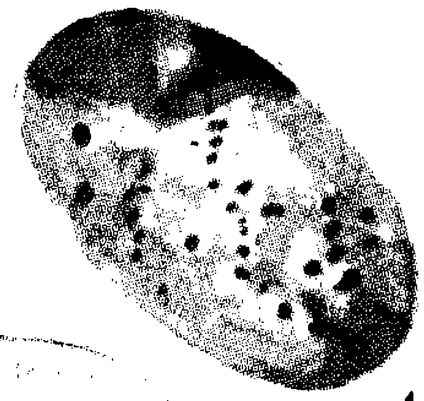
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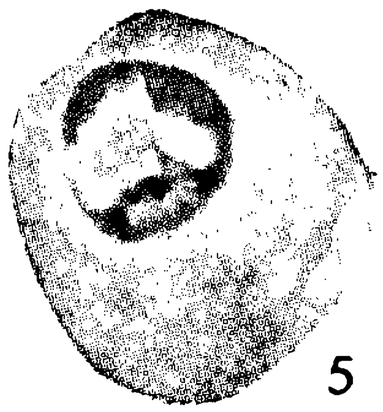
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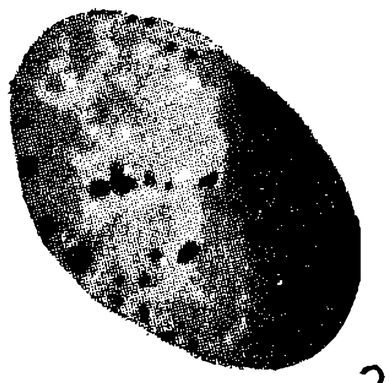
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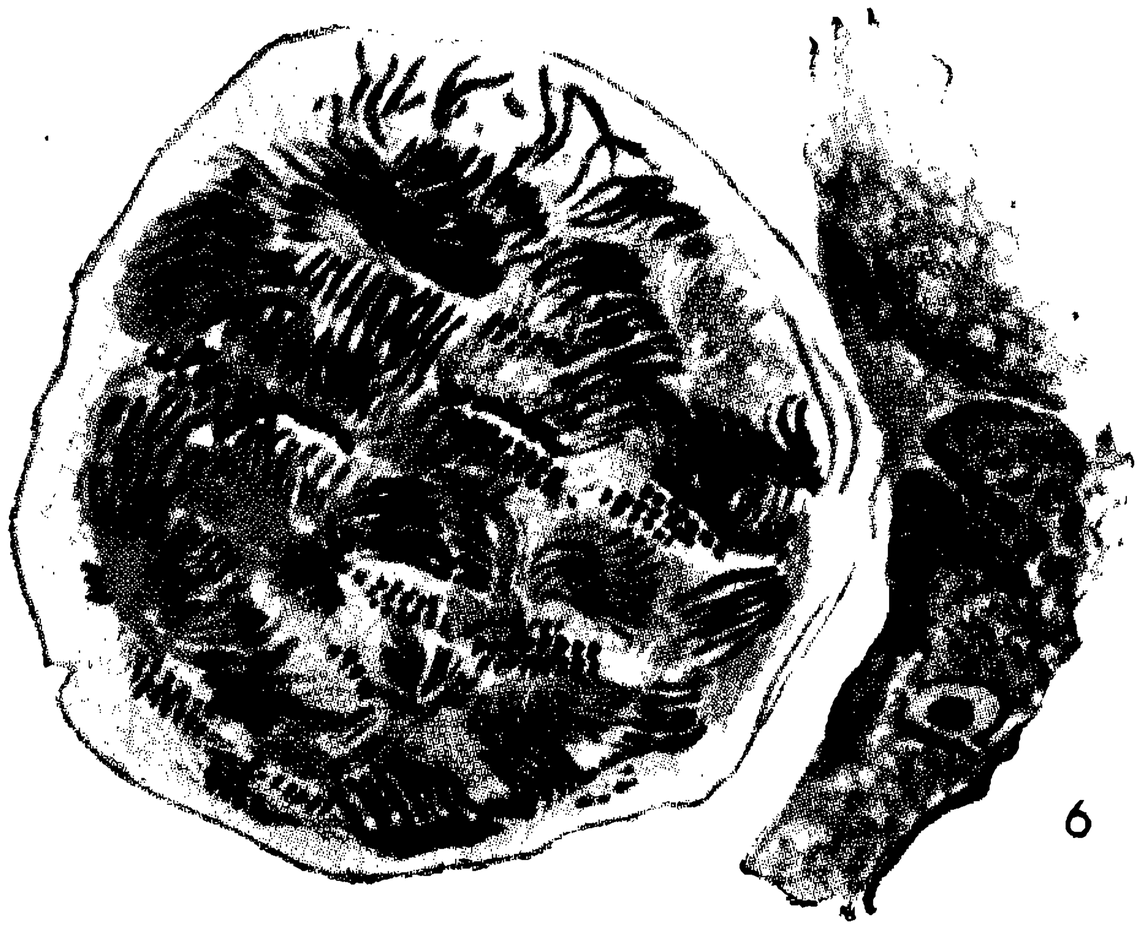
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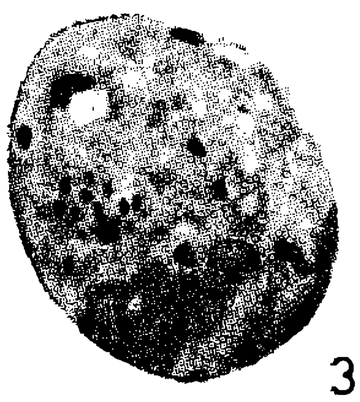
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