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## MÉMOIRES ORIGINAUX

# Parasitic protozoa of the blood of rodents

## III. Two new malaria parasites of Anomalurine flying squirrels of the Ivory Coast

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*Summary*

Stained films of heart blood of 37 specimens of flying squirrels in the Ivory Coast were examined for malaria parasites. Two new species were found in blood films from *Anomalurus peli*. The name *Plasmodium landauae* sp. nov. is proposed for one, and *Plasmodium pulmophilum* sp. nov. for the other. Differences between the new parasites and *P. anomaluri* Pringle, 1960 of *A. derbianus* in Tanzania are discussed.

## *Résumé*

***Protozoaires parasites du sang des Rongeurs : III. Deux nouveaux parasites malariques d'écureuils volants de la Côte d'Ivoire.***

Des frottis de sang de 37 Ecureuils volants ont été examinés en Côte-d'Ivoire, à la recherche de parasites du Paludisme.

Deux espèces de *Plasmodium* ont été trouvées dans le sang d'*Anomalurus peli*. L'une d'entre elles est nommée *Plasmodium landauae* n. sp. et la seconde *Plasmodium pulmophilum* n. sp. Les caractères différentiels entre les nouvelles espèces et *P. anomaluri* Pringle, 1960, parasite d'*A. derbianus* en Tanzanie, sont discutés.


In 1967, Mr Pierre Hunkeler of the Centre Suisse in Abidjan, Ivory Coast, showed me fixed tissues of two flying squirrels which he had collected at Bolo, 50 km north of Sassandra. Blood films from the animals had not been made but the spleen and the liver appeared to be pigmented and when sections of the spleen were later prepared, intracellular malaria pigment was found. I therefore visited the Ivory Coast in October, 1970, to search for malaria parasites of flying squirrels.

In the survey, made in collaboration with Mr Louis Bellier of O.R.S.T.O.M., Centre d'Adiopodoumé, malaria parasites, trypanosomes, haemogregarines and microfilariae were found. A preliminary note of the results has been published (Killick-Kendrick and Bellier, 1971) and a description of the malaria parasites is given in the present paper. Two new species were found : for one the name *Plasmodium landauae* sp. nov. is proposed in honour of M$^{me}$ Irène Laudau and in recognition of her contributions to our knowledge of malaria parasites of African rodents : for the other the name *Plasmodium pulmophilum* sp. nov. is proposed because it was found to have the erythrocytic schizogony largely restricted to the lungs.

## Material and methods.

### Locality.

The survey was carried out in farms and secondary forest near two villages, Guéboua II and Niakousoué (longitude 6° 32" W, latitude 6° 00" N), in the Lakota district of the Ivory Coast. The place was chosen because local hunters said flying squirrels were plentiful. They were hunted mainly in cocoa and coffee farms interspesed wih tall trees left when the land was cleared.

A description of the topography and climate of the locality is as follows. Briefly, it lies at about 200 m. above sea-level in the southern densely forested part of the country. Mean monthly minimum temperatures at Gagnoa, 30 km. away, are 21-22 °C, and mean monthly maximum temperatures range from 28-33 °C. The rainy season begins in March (100-200 mm) and reaches a peak in June (200-300 mm) ;

there is then a lull in July and August (0-50 mm) followed by an increase in September, October and November (50-100 mm). The survey was carried out from 16-23rd of October, towards the end of the rainy season.

FLYING SQUIRRELS.

African scaly-tailed flying squirrels are not true squirrels : they lie in the family Anomaluridae of the suborder Theridomyomorpha (Wood, 1959 ; Simpson, 1959). Two genera, *Anomalurus* and *Anomalurops* form the subfamily Anomalurinae. *Idiurus* and *Zenkerella* are the two genera of the subfamily Zenkerellinae. Descriptions of the forms in West Africa and notes on the little that is know of their habits are given by Rosevear (1969). They are strictly arboreal animals which never normally come to ground. It is thought they are wholly vegetarian, feeding on the fruits, flowers, leaves and bark of trees. They are nocturnal, except for *Anomalurops beecrofti* (Fraser, 1852) which is said to be most active in the late afternoon and early evening. Although this species is known from the Ivory Coast, no specimens were collected during the survey. Almost every female of the three species examined in October, 1970, was pregnant with a single foetus ; the young would have been born at about the end of the rains.

*Anomalurus peli* (Schlegel and Miller, 1885). This animal is found only in the Upper Guinea Forest west of the Dahomey Gap. It is the largest of the anomalurids (fig. 1) ; the weight of the 15 specimens collected in the survey ranged from 1.3-2.0 kg. All were adults which were usually in pairs. Most specimens were shot at night as they fed in the forest canopy, but a few were flushed out of tree holes during the day by banging on the trees.

*Anomalurus derbianus* (Gray, 1842) (= *A. fraseri* Waterhouse, 1843 ; see Rosevear, 1969). The range of this animal is from the Upper Guinea Forest through Central Africa into parts of East Africa. It is a smaller animal than *A. peli ;* the six specimens collected in the Ivory Coast weighed 660-830 gm. They were flushed out of tree holes during the day and shot. They were always solitary, and all were adult.

*Idiurus macrotis* Miller, 1898. This pygmy flying squirrel has a wide but patchy distribution throughout the Upper Guinea Forest, and eastwards across the Dahomey Gap into Central Africa. It is a tiny mouse-like animal weighing less than 30 gm. The 16 specimens collected in the survey were found during the day roosting in hollow trees, frequently in association with the bat *Hipposideros cyclops.* They were smoked out and shot. All were mature.

TECHNIQUES.

Immediately the squirrels were shot, blood was taken from the heart with a syringe and needle. Thick and thin blood films were prepared and, because of the high humidity, were dried with gentle heat. Thin films were fixed momentarily in absolute methanol and were stained on the day of preparation for 45 mins in 10 per cent. Giemsa's stain diluted with phosphate buffered water at pH 7.2. Thick films were simultaneously lysed and stained in 3 per cent. Giemsa's stain at the same pH for 30 mins.

FIG. 1. — *Anomalurus peli*, host of *Plasmodium landauae* sp. nov. and *P. pulmophilum* sp. nov.

**Results.**

THE PREVALENCE OF MALARIA PARASITES.

The results of the survey are given in Table I. Descriptions of the trypanosomes of *Anomalurus* spp. and the haemogregarines of *Idiurus* will be given elsewhere.

**Table I**

Results of the survey of flying squirrels of the Ivory Coast

| | *A. peli.* | *A. derbianus* | *I. macrotis* |
|---|---|---|---|
| *Plasmodium* | 2 | 0 | 0 |
| *Trypanosoma* | 7 (1) | 3 | 0 |
| *Plasmodium & Trypanosoma* | 3 | 2 | 0 |
| *Hepatozoon* | 0 | 0 | 4 |
| Negative | 3 | 1 | 12 |
| Totals | 15 | 6 | 16 |

(1) One was also infected with microfilariae morphologically similar to those of *Onchocercella katangensis* of *Petrodromus tetradactylus* (see Gedoelst, 1916 ; Yorke and Maplestone, 1931) ; the slide is deposited in the collection of Dr Ralph Muller, Dept of Medical Helminthology, London School of Hygiene and Tropical Medicine.

No malaria parasites were found in 16 *I. macrotis,* the spleens of which were apparently normal with no sign of enlargement or pigmentation. Two typical spleens measured 16 × 2 mm and 20 × 1.5 mm. Patent infections of malaria parasites were found in 5/15 *A. peli* and 2/6 *A. derbianus.* All specimens, however, had enlarged and pigmented spleens suggesting that they were or had been infected. Measurements of the spleens of 7 *A. peli* are given in Table II. The spleens of one specimen of *A. derbianus* (which is a smaller animal than *A. peli*) measured 35 × 10 mm ; this animal (No 70/34) had patent infections of malaria parasites and trypanosomes.

**Table II**

Spleen sizes of 7 *Anomalurus peli* with patent infections of *Plasmodium* (P), *Trypanosoma* (T) or both

| Spleen size in mm. (length × maximum width) | Infection | | Survey number |
|---|---|---|---|
| 75 × 28 | P | T | 70/25 |
| 70 × 23 (2) | P | T | 70/37 |
| 65 × 22 | P | T | 70/35 |
| 65 × 22 | | T | 70/24 |
| 60 × 20 | P | T | 70/22 |
| 60 × 18 | | T | 70/52 |
| 55 × 25 | P | | 70/23 |

(2) Thickness was 12 mm ; weight was 6.7 gm.

The stages of malaria parasites seen in thin films of the heart blood of the 5 *A. peli* and 2 *A. derbianus* with patent infections are listed in Table III. Four out of 13 specimens of *A. peli* collected at Géboua II had very light infections, whereas the only 2 collected at Niakousoué had moderately heavy infections and provided material for the descriptions given below. These animals were a breeding pair and were shot in the same tree during the day. They were only slightly wounded, but the female, No 70/35, collapsed an hour later and died as blood was collected. The male, No 70/37, was taken alive to Abidjan and maintained overnight in captivity. Although its wounds were slight, it was in a poor condition and the next day it was sacrificed. Blood, taken from the heart with heparin as an anticoagulant, was inoculated intraperitoneally into 7 white mice, 3 of which were splenectomized on the following day. Smears prepared from the lungs, liver, spleen, kidney, heart muscle, skeletal muscle and bone-marrow of the flying squirrel were fixed in methanol and stained in Giemsa's stain.

All six specimens of *A. derbianus,* two of which had patent infections of malaria parasites, were collected at Niakousoué. The parasitaemias were so low that the parasites cannot be fully described and given an identity. A single schizont seen in a film from *A. derbianus* No 70/65 had 16 nuclei and caused no reddening of the host-cell.

**Table III**

Stages of malaria parasites seen in the heart blood of infected anomalurines

| Species and survey number | Stages seen in thin smears of heart blood. | Place collected |
|---|---|---|
| *A. peli* 70/22 | 1 microgametocyte | Guéboua II |
| *A. peli* 70/23 | Few rings. | » |
| *A. peli* 70/25 | Very few rings. | » |
| *A. peli* 70/35 | Rings, trophozoites, immature schizonts, rare macrogametocytes. | Niakousoué |
| *A. peli* 70/37 | Rings, trophozoites, rare immature schizonts. | » |
| *A. peli* 70/42 | One ring. | Guéboua II |
| *A. derbianus* 70/34 | One trophozoite. | Niakousoué |
| *A. derbianus* 70/65 | One trophozoite.<br>One schizont. | » |

DESCRIPTIONS OF THE MALARIA PARASITES OF A. PELI.

Such striking differences were found in the morphology of the parasites of the specimens of *A. peli* collected at Niakousoué that it is clear they comprised two different species.

***Plasmodium landauae*** sp. nov., in the blood of *A. peli* No 70/35 (fig. 2).

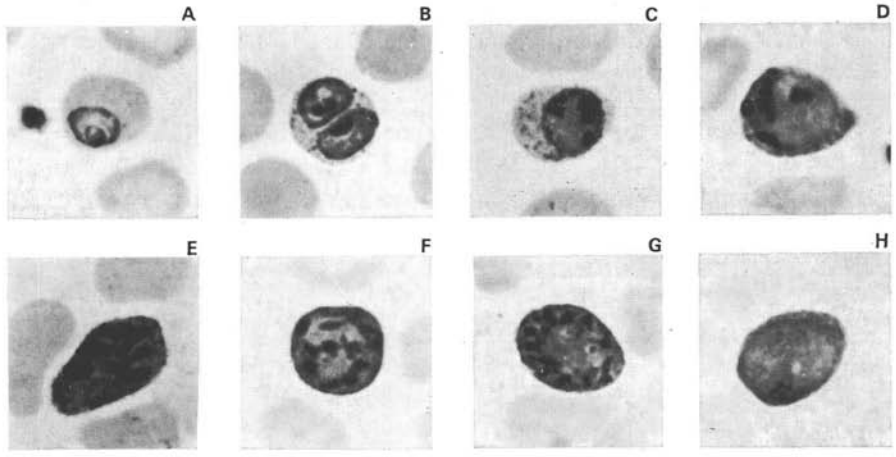


FIG. 2. — *Plasmodium landauae* sp. nov. A : trophozoite ; B : rare double infection ; C : young schizont ; D-G : schizonts ; E : macrogametocyte. Giemsa-stained smear of heart blood. X : 1,800

All parasitized cells appeared to be normocytes, the diameter of which was 7-8 μm. The youngest parasites were round or oval ring forms lying eccentrically in the host cell and measuring 2 μm in diameter. The nucleus was peripheral and lay within the smooth outline of the parasite ; twin nuclear masses were never seen.

Trophozoites were round or oval and compact, with no sign of amoeboidicty (fig. 2A). The cytoplasm stained blue, with a darkly staining rim. On reaching a diameter of about 4 μm, fine grains of golden pigment lay scattered around the edge of the parasite. Only one infected cell was seen with two parasites, both late trophozoites at the same point of growth (fig. 2B).

Although some infected cells were finely stippled (fig. 2C), this was probably not caused by the parasite since similarly stippled but uninfected cells were common in the material examined. The nuclei of asexual forms began to divide as the diameter of the parasite reached 5μm (fig. 2C). The infected cell then began to stain a dark red colour similar to but even more striking than that of Landau's (1965) freshly isolated strains of *P. vinckei chabaudi*. A few darkly red staining erythrocytes were found in all thin films from *A. peli* but since they were never infected with rings or trophozoites, the deep colour of the host cells of schizonts is presumed to be a change caused by the growing parasite and not simply a normal colour ; all schizonts were

in reddened cells (fig. 2C to G). The maximum number of nuclei of the schizonts was 12 (fig. 2F, G) ; one had nuclei budding off at the periphery of the parasite (fig. 2G). Pigment in the late but still not quite mature schizonts was clumped into one or two golden masses which were strongly birefringent under polarized light. The host cells of these forms were slightly enlarged with a mean diameter of up to 9 μm. Fully mature schizonts were not found and the number of merozoites produced is not known ; it must, however, be 12 or more.

Two oval macrogametocytes in hypertrophied red cells measured 9 × 6.5 μm. Along one edge of the parasites, a reddened rim of the host cell was visible (fig. 2H). The morphology of the nuclei was unusual ; they stained the usual red colour, but had the appearance of a cluster of darkly staining dots. The pigment of the gametocytes lay scattered throughout the blue cytoplam in fine golden grains. No male gametocytes were found.

***Plasmodium pulmophilum*** sp. nov. in the blood of *A. peli* No 70/37 (fig. 3).

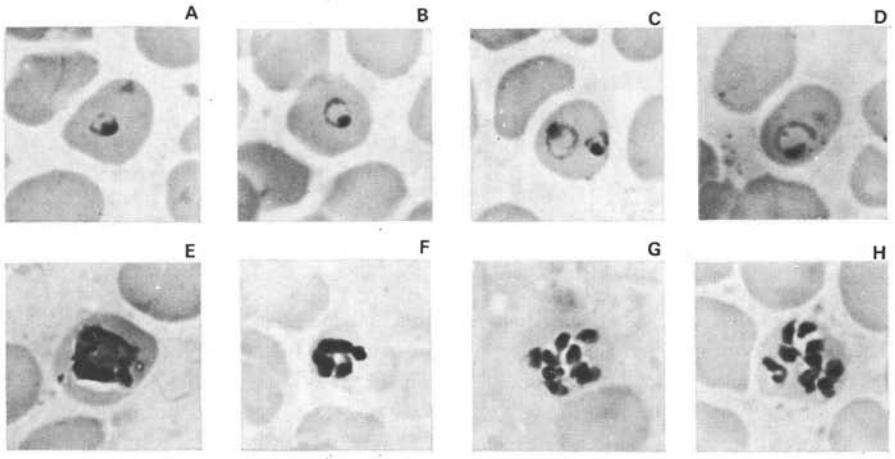


FIG. 3. — *Plasmodium pulmophilum* sp. nov. A-B : ring forms ; C : common double infection ; D : trophozoite ; E : angular shaped developing schizont ; F-H mature schizonts. Giemsa-stained lung smear. X : 1,800

With the exception of a single schizont and rare trophozoites, the only malaria parasites seen in a search of five films of heart blood from this animal were ring forms. Two of the films were made on the day of collection, and three on the next day. The rings were round with a thin wisp of blue cytoplasm and normally lay near the centre of the host cell. The nucleus stained deeply and appeared as a single round or oval dot lying at the periphery, sometimes protruding slightly (fig. 3 A to C). Cells with double infections were not uncommon (fig. 3C).

Later stages were easily found in the smear of the lung (which was the main site of the erythrocytic schizogony) and to a lesser degree that of the spleen, but not in

smears of heart and skeletal muscle, bone-marrow, liver or kidney. Trophozoites were either simply large rings (fig. 3D) or, as they grew larger, compact round bodies. Their nucleus was oval and fine, scanty, almost invisible pigment lay at the edge of the cytoplasm. The nucleus began to divide after the trophozoites had grown to a diameter greater than half that of the host cell. Immature schizonts tended to be angular in shape (fig. 3E) and their nuclei stained very deeply ; lighter patches of red material lay in the blue cytoplasm. Comparatively little pigment was visible at this stage, tending to lie in separate tiny grains at the periphery of the parasite. It was birefringent under polarized light, and formed into one or two golden clumps just before the schizont became fully mature. Mature schizonts produced 6-10 merozoites (fig. 3E to H) but the commonest number, seen in more than three-quarters of the mature forms, was eight. The merozoites were angular or round bodies with a deeply staining nucleus against which lay a tiny wisp of blue cytoplasm (fig. 3H). The parasites caused no enlargement, stippling or dark colouration of the host cell, but red cells containing mature schizonts tended to stain a little more palely than uninfected cells. No gametocytes of either sex were seen.

Intact and splenectomized mice inoculated with heparinized heart blood failed to become infected.

**Discussion.**

The malaria parasites of *Anomalurus* in the Ivory Coast lie in the subgenus *Vinckeia* Garnham, 1964, in which are grouped 15 species of *Plasmodium* of mammals other than the supralemuroid primates (Garnham, 1966 ; Lien and Cross, 1968). In the definition of the subgenus, the erythrocytic schizonts are described, with some exceptions, as small with 8 merozoites or less. There are, however, too many exceptions for this character to be considered typical of the subgenus. Of the 15 named species of *Vinckeia,* the schizonts of 7 are large and have more than 8 merozoites (*P. cephalophi* and *P. brucei* of duiker ; *P. voltaicum* of a bat ; *P. anomaluri* of *Anomalurus ; P. watteni* of *Petaurista ;* and *P. berghei* and *P. vinckei* of African murine rodents). The new parasites from the Ivory Coast are also exceptions ; both have large schizonts, and those of one produce 12 or more merozoites.

The parasites of *A. peli* may be differentiated, one from the other, by the erythrocytic schizonts. Those of *P. landauae* caused a deep reddening and slight enlargement of the host cell and produced not less than 12 merozoites, whereas the schizonts of *P. pulmophilum* changed the host cell very little and produced 8 merozoites (range 6 - 10). No gametocytes of *P. pulmophilum* were found in the blood films from *A. peli* No. 70/37 and until they are seen it is not known if the cluster of points which comprised the nucleus of the macrogametocytes of *P. landauae* is a useful differential character.

From a comparison of the Ivory Coast parasites with *P. anomaluri* Pringle, 1960 of *A. derbianus* of Tanzania, it is concluded that the parasites of *A. peli* are new (see Table IV). *P. anomaluri* differs from *P. landauae* by the presence of double nuclear masses in some rings of the former species, the morphology of its trophozoites, the lack

**Table IV**

Differences between three malaria parasites of African flying squirrels

| | *Plasmodium anomaluri* (3) | *Plasmodium landauae* | *Plasmodium pulmophilum* |
|---|---|---|---|
| Vertebrate host | *Anomalurus derbianus* | *Anomalurus peli* | *A. peli* and possibly *A. derbianus* |
| Locality | Tanzania | Ivory Coast | Ivory Coast |
| Ring forms | sometimes with 2 nuclear masses | never with 2 nuclear masses | never with 2 nuclear masses |
| Trophozoites | a big ring, or compact, or amoeboid often with 2 or more vacuoles | compact or with 1 vacuole, never amoeboid | compact or with 1 vacuole, never amoeboid. |
| Number of merozoites | 8 or more | 12 or more | normally 8, range 6-10 |
| Gametocytes | nucleus with a « karyosome » | nucleus of ♀ a cluster of points | ? |
| Colour of pigment | brown | golden | golden |
| Affect on host cell | unaffected | deep red as parasite grows ; cell enlarged. | slightly pale, cell not enlarged |
| Multiple infections | absent | rare | common |

(3) From the description of Pringle (1960).

of reddening of infected host cells, the colour of the pigment and the appearance of the nuclei of the macrogametocytes. It differs from *P. pulmophilum* by the absence of double infections in *P. anomaluri,* the presence of double nuclear masses in some rings of the latter parasite, the morphology of the trophozoites, the colour of the pigment and probably by a larger number of merozoites produced by mature schizonts of *P. anomaluri* (which Garnham (1966) believed to be more than eight).

The apparent restriction of the erythrocytic schizogony of *P. pulmophilum* mainly to the lungs is an important distinguishing feature if it proves to be constant. Mr R. L. Jacobsen (personal communication) examined lung smears from one of three specimens of *A. derbianus,* all infected with *P. anomaluri,* which he collected in the type locality in Tanzania. No schizonts were present, and the preferred site of schizogony for this parasite remains unknown. Pringle (1960) found a few schizonts in heart blood, but none in the peripheral blood.

The parasitaemias of the only two infected *A. derbianus* were so low that identifications were not possible. The parasites of these animals may have been *P. anomaluri.* It seems more likely, however, that they were one of the two parasites of *A. peli,* but the single schizont seen in the blood of *A. derbianus* 70/75 had 16 nuclei and caused no reddening of the host cell ; this slender evidence suggests that the parasites of *A. derbianus* may have been neither of those of *A. peli.*

In the Ivory Coast, anomalurine malaria parasites occur in more than one locality. Soon after I had found pigment in the spleens of *Anomalurus* from Bolo in 1967, Mr Hunkeler returned there, collected two specimens of *A. peli* and prepared blood films ; in 1970 Mr Hunkeler sent me the films. One was negative, but the other was heavily infected with a malaria parasite believed to be *P. pulmophilum*. Almost all the parasites were trophozoites but a few very small rings were seen and, after many hours search, a few immature schizonts were found. Even after re-staining, they caused no reddening of the host cell. No gametocytes were seen.

The results of the survey in Lakota district, together with the infections found by Hunkeler in another part of the Ivory Coast and Pringle and Jacobsen in Tanzania, suggest that African flying squirrels are common hosts of malaria parasites. Rodhain *et al.* (1913) and Schwetz (1933) examined a few in the Republic of Zaire, but did not record malaria parasites. Since parasitaemias are commonly very low, these reports should not be taken to suggest the absence of malaria parasites of flying squirrels in that part of Africa. Apart from those of the Belgian workers and workers in the Ivory Coast and Tanzania, there appear to be no reports of the examination of blood smears of anomalurine rodents. No parasites of these animals of any kind are listed in Bray's (1964) checklist of parasitic protozoa of West Africa. This is not because flying squirrels are scarce, but probably because they are overlooked ; they are nocturnal : they are seldom hunted for food : and they are not exhibited in zoos because they are extremely difficult to keep in captivity.

The transmission of the parasites of *Anomalurus* warrants a special study. Since the animals are strictly arboreal, the natural vector is presumably an anopheline which lives in the forest canopy or in tree holes. There are no known canopy-dwelling *Anopheles* in Africa, but further work may reveal an African anopheline with arboreal habits similar to those of *Anopheles cruzi,* the vector of *P. brasilianum* of monkeys in Brazil (Deane *et al.,* 1971). Until the vectors of the anomalurine malaria parasites are discovered, the possibility that sporozoites of unknown identity in wild-caught silvatic anophelines in Africa may be those of a parasite of flying squirrels must be borne in mind.

**Diagnosis : *Plasmodium landauae*** sp. nov. Blood stages. No predilection for immature erythrocytes ; polyparasitim rare ; ring forms never with twin nuclear masses ; no accessory dot ; trophozoites compact or with one vacuole, never amoeboid ; schizonts nearly fill host-cell and produce 12 or more merozoites ; nucleus of macrogametocyte appears as a cluster of points ; as asexual parasites grow, infected erythrocytes become deep red in colour and slightly enlarged ; gametocytes cause the same reddening and greater enlargement ; pigment is golden yellow.

Sporogonic stages. Unknown.

Exoerythrocytic stages. Unknown.

Hosts. Type vertebrate host is *Anomalurus peli* (Schlegel and Miller, 1885) (Rodentia : Anomaluridae) ; invertebrate host unknown.

Geographical range. Believed to be restricted to the Upper Guinea Forest, west of the Dahomey Gap, in West Africa. Type locality is secondary forest

and farmland near Niakousoué, Lakota District, Ivory Coast (longitude 6° 32" W, latitude 6° 00" N).

Type material will be deposited in the Wellcome Museum, London.

**Diagnosis : *Plasmodium pulmophilum*** sp. nov. Blood stages. No predilection for immature erythrocytes ; polyparasitism common ; ring forms never with twin nuclear masses ; no accessory dot ; trophozoites compact or with one vacuole, never amoeboid ; schizonts rare in the peripheral and heart blood, and erythrocytic schizogony largely confined to the blood capillaries of the lungs and, to a lesser degree, the spleen ; mature schizonts almost fill the host-cell, tend to be angular in shape and produce 8 merozoites, range 6-10 ; as parasites grow, infected cells stain palely but are unenlarged ; pigment is golden yellow ; gametocytes unknown.

Sporogonic stages. Unknown.

Exoerythrocytic stages. Unknown.

Hosts. Type vertebrate host is *Anomalurus peli* (Schlegel and Miller, 1885) Rodentia : Anomaluridae) ; *Anomalurus derbianus* (Gray, 1842) is a possible host ; intact and splenectomized laboratory mice insusceptible ; invertebrate host unknown.

Geographical range. As far as is known, the same as that of *P. landauae*.

Type material will be deposited in the Wellcome Museum, London.

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