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MÉMOIRES ORIGINAUX

Parasitic Protozoa of the blood of Rodents

V. *Plasmodium vinckei brucechwatti* subsp. nov.

A malaria parasite of the thicket rat, *Thamnomys rutilans*, in Nigeria

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Summary.

A description is given of the blood stages of a new subspecies of *Plasmodium vinckei* in the blood of naturally infected thicket rats (*Thamnomys rutilans*) from Nigeria and experimentally infected mice. Sporogony was obtained at 25 °C in *Anopheles stephensi*, *A. quadrimaculatus* and *A.l. atroparvus*, but sporozoites in the salivary glands of the mosquitoes were never infective. The new parasite is differentiated from 7 other species of malaria parasites of African rodents principally on the morphology of the erythrocytic stages. It is designated as a subspecies of *P. vinckei* (*P.v. brucechwatti* subsp. nov.) because of the (i) zoogeography, (ii) morphology of sporogonic stages and (iii) forms of isoenzymes of blood stages.

Résumé.

Protozoaires parasites du sang de Rongeurs : V. *Plasmodium vinckei brucechwatti* subsp. nov., parasite du paludisme du Rat arboricole *Thamnomys rutilans* au Nigeria.

Description des stades sanguins d'une nouvelle espèce de *Plasmodium vinckei* chez des *Thamnomys rutilans* du Nigéria et des souris blanches infectées expérimentalement. La sporogonie a évolué à la température de 25 °C chez *Anopheles stephensi, A. quadrimaculatus* et *A. l. atroparvus,* mais les sporozoïtes dans les glandes salivaires n'ont jamais été infectants. Le nouveau parasite a été différencié de 7 autres espèces de *Plasmodium* de Rongeurs africains essentiellement sur la morphologie des stades érythrocytaires. Il est considéré comme une sous-espèce de *P. vinckei* (*P. v. brucechwatti*) en raison de différences : 1) dans la zoogéographie ; 2) la morphologie des stades sporogoniques ; 3) l'analyse des isoenzymes des formes sanguines.

Bruce-Chwatt and Gibson (1955) briefly described a malaria parasite isolated in Nigeria from a rodent identified as *Praomys tullbergi.* Blood pooled from several wild rodents had been inoculated into a white mouse which was later found to have parasites in the blood. By the time the parasites were seen, the blood of the mouse had become negative and the strain was lost. Over 1,000 specimens of *Praomys* were then trapped in the same locality and their blood was again inoculated into mice. No more malaria parasites were found (Bruce-Chwatt and Gibson in Killick-Kendrick, 1973 *a*).

In 1967 I visited Western State, Nigeria, and examined rodents from the locality. No malaria parasites were found in 20 *Praomys,* but 6 out of 18 thicket rats, *Thamnomys rutilans,* were infected with a *vinckei*-like parasite apparently the same as that of Bruce-Chwatt and Gibson (Killick-Kendrick, Shute and Lambo, 1968). One thicket rat was additionally infected with *P. yoelii nigeriensis* Killick-Kendrick, 1973, a parasite of the *berghei*-group (Killick-Kendrick, 1974 *b*). Two strains (Nos N 48 and N 52) of the *vinckei*-like parasite of thicket rats were established in laboratory mice (Killick-Kendrick *et al.,* 1968). In January 1969, a third strain (No 1/69) was isolated from a specimen of *Thamnomys rutilans* sent from Lagos, Nigeria, to London, England, by Mr A. O. Lambo.

Between 1967 and 1972 I studied the erythrocytic and sporogonic stages of the new parasite and vainly attempted to demonstrate exoerythrocytic schizonts. Because some stages of the life-cycle had not been seen, it was at first thought unwise to name the Nigerian parasite. However, it has now been shown that it can be distinguished from related parasites by the forms of enzymes of the blood stages (Carter, 1974 ; Carter and Walliker, 1975). Increasing confidence in this biochemical approach to the taxonomy of murine parasites (Killick-Kendrick, 1974 *b*) and the recognition of the probable influence of ecological barriers in West Africa on speciation in the related *berghei* group (Killick-Kendrick, 1973 *a*), have

led to the view that formal taxonomic recognition should now be given to the *vinckei*-like parasite of Nigeria. It is here named *Plasmodium vinckei brucechwatti* subsp. nov. in honour of Prof. L. J. Bruce-Chwatt, who was joint discoverer of the parasite and who encouraged me in this work.

In the present paper, descriptions are given of erythrocytic stages of *P.v. brucechwatti* in naturally infected thicket rats and experimentally infected mice, and of the sporogonic stages in laboratory bred *Anopheles*. A preliminary note, details of the materials and methods, and accounts of the type locality and the isolation of strains are given elsewhere (Killick-Kendrick *et al.*, 1968 ; Killick-Kendrick, 1970, 1971, 1973 *a*).

Erythrocytic stages of *P.v. brucechwatti* subsp. nov. in thicket rats.

All 6 of the naturally infected thicket rats had patent infections of *P.v. brucechwatti* (see Plate II b in Killick-Kendrick, 1971). Four of the 6 had high parasitaemias, and in thin blood films from the rat with the heaviest infection there were 1-3 infected cells in each field (× 100 obj.). All stages were present, and the plate and the following description are based on material from this rat (No. N 48).

The parasite had no special affinity for immature red cells. Infected erythrocytes were neither stippled nor, with few exceptions, enlarged but the host-cell was much distorted by the asexual stages; by the time 6 nuclei had formed it was a misshapen, palely staining rim around the parasite (*fig. 9, plate 1*).

The smallest rings had a diameter of 1 μm. Their nuclei, which were round and stained deeply, were commonly divided into two equal masses or, more rarely, into three with one mass smaller than the other two. The cytoplasm was a pale blue wisp of material lying close to the nucleus. Double infections were occasionally seen, and a few cells were found with three parasites (*fig. 1, 2, 3; plate 1*). Cells with multiple infections were often enlarged.

With growth, a vacuole appeared and the parasite then adopted the typical signet-ring shape. The nucleus of the young trophozoite often lay within the vacuole which sometimes persisted until the parasite reached 3.5 μm in diameter, i.e. half the diameter of the cell. At this point of growth very fine pigment, typically peripheral, first became visible. Trophozoites were never markedly amoeboid, and further growth was accompanied by a progressive reduction in the size of the vacuole until it completely disappeared leaving the late trophozoite as a round compact body (*fig. 4, 5; plate 1*). The twin nuclear masses of some rings persisted in the trophozoite which then simulated a young schizont. The nucleus of the growing form often lay in a vacuole and was oval or bar-or bean-shaped, but seldom round. A few trophozoites were band forms. Nuclear division of schizonts did not begin before the diameter of the parasite reached half that of the host cell and late trophozoites were therefore indistinguishable from immature gametocytes.

Only few schizonts were present in the peripheral blood. The smallest had two nuclei and was a round compact body, with a few cytoplasmic extrusions, occu-

pying a little more than half the diameter of the host-cell (*fig. 6; plate 1*). Nuclei of developing schizonts were irregular in shape or round or bar-shaped, and often had a strongly staining portion resembling an eccentric nucleolus. The cytoplasm stained blue, and usualy contained one or two small, indistinct vacuoles (*fig. 7, 8, 9; plate 1*). Pigment in schizonts became progressively more abundant as the parasites grew, and was typically peripheral. It clumped into a single yellow mass before the merozoites were fully formed.

Schizonds produced 8-12 merozoites. One, free from its host-cell, was composed of eight irregularly shaped merozoites each measuring about 1 μm across (*fig. 10; plate 1*).

Fully grown female gametocytes were spherical and measured 7-8 μm in diameter. The host-cell was not visible and was completely filled by the parasite. The nucleus, which was small and peripheral, stained a deep purplish red. The cytoplasm was the typical blue colour, and contained a variable number of separate grains of fine yellow pigment (*fig. 11; plate 1*). Only two male gametocytes were found. They were the same size as the macrogametocytes, but were palely staining pink bodies with a pale pink peripheral nucleus about twice the size of that of the female form. Up to twenty grains of fine yellow pigment were scattered through the cytoplasm (*fig. 12; plate 1*).

Erythrocytic stages and course of infection of *P.v. brucechwatti* subsp. nov. in white mice.

The morphology of *P.v. brucechwatti* in the blood of white mice differed little from that seen in thicket rats. Infected cells were neither stippled nor enlarged, and there was no reddening of parasitized cells as seen in infections of some other members of the *vinckei* group (Landau, 1965; Landau, Michel, Adam and Boulard, 1970; Carter and Walliker, 1975). As in the thicket rat, infected cells became much distorted by the time asexual stages were half grown. Other points of similarity were the presence of double nuclei in many ring forms; the frequency of double infections; the compact nature of trophozoites as the central vacuole was lost; and the general peripheral position of pigment which was in fine yellow grains until it clumped in schizonts before they were fully mature. In mice, most schizonts produced twelve merozoites, with a range of 8-14 (compared to 8-12 in thicket rats). Sixteen merozoites were occasionally seen, but these were thought to have been in cells with double infections.

Gametocytes were round, 6-7 μm in diameter, and completely filled the host cell the rim of which was occasionally visible as a tattered fringe. The morphology of the few in the blood of thicket rats was confirmed by the appearance of the gametocytes in mice. The nuclei of both males and females were always peripheral; that of the female was small and stained deep red, whereas that of the male was more diffuse and stained pink. The cytoplasm of the macrogametocyte was bright blue, and contained specks of yellow pigment. That of the microgametocyte stained pink, but abundant fine pigment tinted the cytoplasm to the colour of a biscuit.

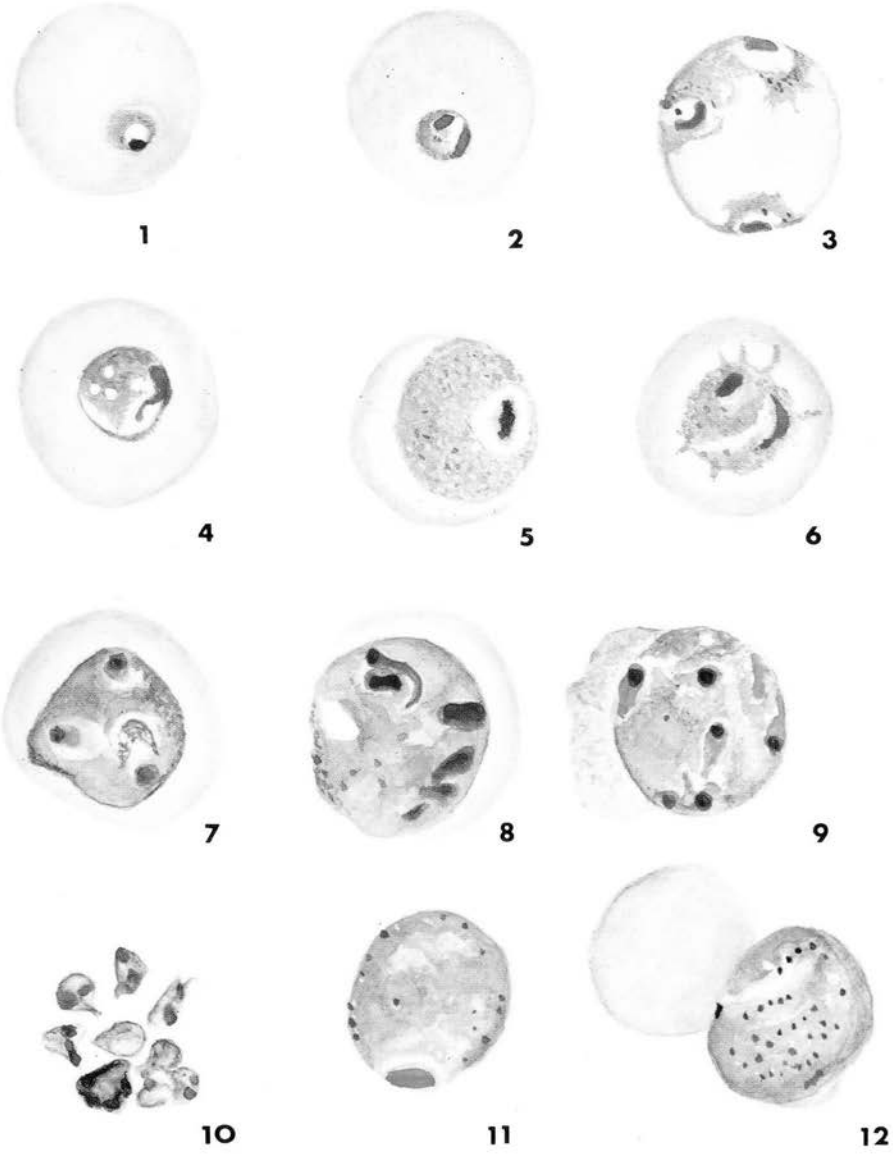

Plate I. — *Plasmodium vinckei brucechwatti* subsp. nov. in the blood of naturally infected *Thamnomys rutilans* (Nº N48) ; X 3,000 ; J.V. Scorza pinx.

Parasitaemias in mice with blood-induced infections became high by the tenth day. Although most mice inoculated with blood from *Thamnomys* survived the infection with a spontaneous recovery between days 15 and 25, after only three consecutive blood passages the virulence was enhanced, and mice then seldom lived longer than two weeks. Gametocytes were most numerous as the parasitaemia declined towards recovery.

There were no detectable differences in the morphology of the blood stages of the three strains studied, nor in the course of infection in mice.

Sporogony of *P.v. brucechwatti*.

Light infections of the salivary glands of laboratory bred *Anopeles stephensi, A. quadrimaculatus* and *A. labranchiae atroparvus* were obtained but sporozoites were never infective. The temperature of incubation was 24-26 °C, i.e. the optimum temperature for the sporogony of the sympatric parasite *P. yoelii nigeriensis*. The harvest of sporozoites from the salivary glands of infected mosquitoes was always low and rarely exceeded 300. In contrast, the minimum harvest of sporozoites of *P.y. nigeriensis* in *A. stephensi* is 2,500 per mosquito (Killick-Kendrick, 1973 *a*).

No oocysts were found on the stomachs of *A. gambiae* B fed with a control batch of *A. stephensi* on an infected multimammate rat (*Table I*). In *A. quadrimaculatus*, the numbers of oocysts on midguts were similar to those in a control batch of *A. stephensi*, but fewer *A. quadrimaculatus* became infected ; on day 15 there were Ross' black spores on some midguts of *A. quadrimaculatus*, a phenomenon not seen in *A. stephensi*. In *A.l. atroparvus* oocyst rates were lower than in control batches of *A. stephensi*, and there were significantly fewer oocysts in the former mosquito. It is concluded that none of the species of mosquitoes was a suitable laboratory host (*Table I*).

A description of the sporogonic stages in *A. stephensi* is given below. With other gametocyte carriers, species of mosquito or temperatures of incubation a healthier sporogony would be indicated if (i) oocysts grew more evenly, (ii) fewer oocysts died, (iii) larger numbers of sporozoites invaded the salivary glands or, (iv) the sporozoites were infective.

The youngest oocysts seen were in fresh preparations of midguts three days after infective feeds. They were always markedly oval. Abundant pigment, which was a striking feature, lay in loose masses and short lines which were sometimes arranged as a fan (*fig. 1*). The mean diameter of 10 3-day oocysts was 9.5 μm (8.2-10.8 μm).

By day 5, the diameter of the oocysts had increased to 16 μm (11-19 μm; 20 oocysts). They were now spherical and pigment was still visible. On day 7 the oocysts measured 27 μm (20-33 μm; 20 oocysts), and on day 8, 32 μm (24-43 μm; 20 oocysts). There was still no differentiation of the contents of the oocysts. In some of the largest, pigment was often visible lying in one more loose masses, but not in lines.

TABLE I. — Sporogony of *P.v. brucechwatti* in four species of *Anopheles* (at 24-26 °C)

Mosquitoes	Gametocyte carriers	Days of infection when mosquitoes fed	Oocyst rates %	Numbers of oocysts	Recipients (1) of sporozoites harvested from salivary glands	Doses of sporozoites
A. stephensi (28 batches)	mice ; *Praomys natalensis*	various	66-90	1-300	mice *Grammomys surdaster*	various various
A. gambiae B	*P. natalensis*	12	0	0,	—	—
A. stephensi			80	2-166	—	—
A. quadrimaculatus	Mouse	8	60	same	2 mice	5,850
A. stephensi			83			
A.l. atroparvus	Mouse	8	25	< 21	*G. surdaster*	10,500
A. stephensi			63	23-300	*G. surdaster*	14,000 (2)
				→	Mouse	7,000
A.l. atroparvus	Mouse	9	50	< 33	Mouse	6,500 (3)
A. stephensi			83	> 200 →		

(1) No recipients became infected.
(2) Pooled from both batches of *A.l. atroparvus*.
(3) Pooled from control batches of *A. stephensi*.

On day 9, there were the first signs of the formation of sporozoites, with many of the larger oocysts having finger-like processes. The growth of some oocysts then stopped and after day 9 many apparently dead oocysts measuring about 30 μm in diameter were seen on all midguts. Under phase-contrast illumination, dead oocysts were yellowish, and pigment was sometimes visible in small clumps scattered through the parasite with no recognizable pattern. The mean diameter of six oocysts within which sporozoites were forming on day 9 was 47.5 μm (40-53 μm). Wheel-like masses of forming sporozoites as in oocysts of *P. berghei* (Yoeli, 1965) and *P. yoelii* (Wéry, 1968) were not seen.

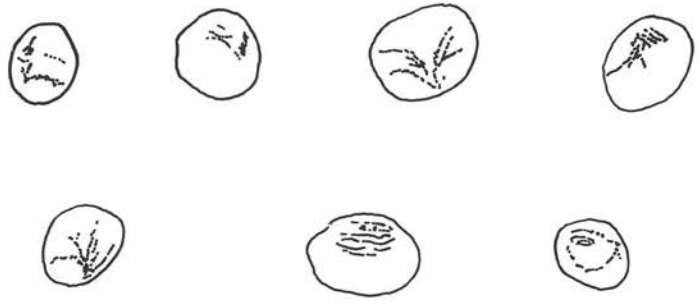

FIG. 1. — Pigment patterns of oocysts of *P.v. brucechwatti* subsp. nov. on the midguts of experimentally infected *A. stephensi* (25 °C); day 3; fresh preparation; × 1,500.

The first fully mature oocysts were found on day 11, but maturity was usually delayed until a day later. The sizes of mature oocysts varied from 40-70 μm with a mean of 54 μm ; the extremes were only rarely encountered, and the normal range was 50-60 μm. Sporozoites were in the glands of a few mosquitoes on day 12, but in the majority invasion of the salivary glands took place on day 13. The day of invasion was related to the numbers of oocysts on the midguts of a batch with the shorter time associated with only few oocysts.

The sporozoite rate was always the same as the oocyst rate and as long as oocysts were established on the midgut, sporozoites were certain to be produced and to invade the salivary glands. But even when hundreds of oocysts were present, heavily infected glands were never obtained. It was usually necessary to crush the glands before sporozoites could be seen.

In stained smears of crushed salivary glands the sporozoites were slender bodies with the nucleus lying a little away from the centre of the parasite (*fig. 2*). One end of the sporozoites stained more strongly than the other, and if it is assumed that this was the anterior end with its apical complex, the nuclei lay posterior to the centre. The mean length of 50 sporozoites in a stained smear of the salivary glands of *A. stephensi* was 14.7 μm (S.E. 0.5 μm) with a range of 10.0-24.0 μm.

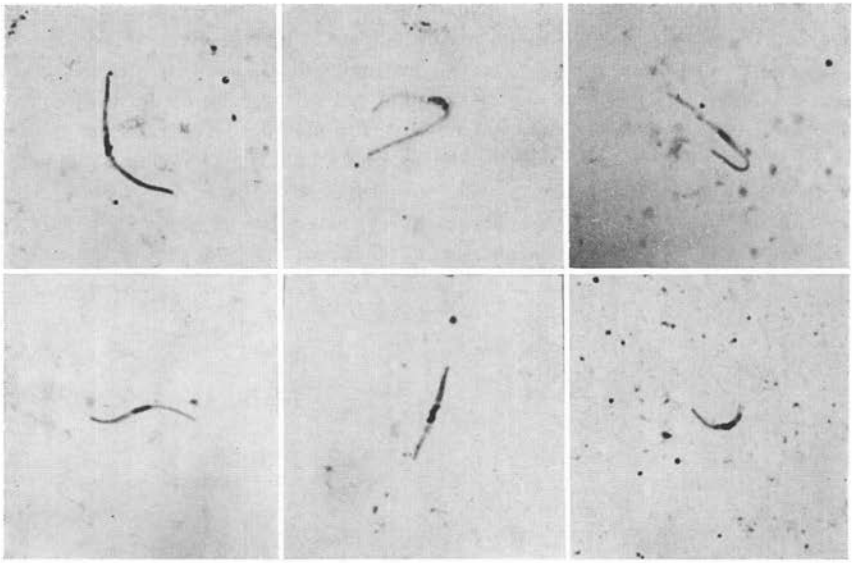

FIG. 2. — Sporozoites of *P.v. brucechwatti* subsp. nov. in a stained smear of the salivary glands of experimentally infected *A. stephensi* (25 °C); × 1,500.

Discussion.

Bruce-Chwatt and Gibson (1955) were the first to describe a *P. vinckei*-like parasite from a Nigerian rodent identified as *Praomys tullbergi*. A redescription was given by Garnham (1966). Prof. Garnham kindly lent me the only remaining slide of Bruce-Chwatt and Gibson's parasite, made from a subinoculated mouse, and I have compared it with *P.v. brucechwatti* isolated 13 years later in the same locality from *Thamnomys rutilans*. There were the following points of similarity: (i) infected cells were neither enlarged nor stippled, (ii) there was no predilection for immature erythrocytes, (iii) multiple infections were occasionally seen, (iv) rings often had double nuclei, (v) trophozoites were round compact bodies with peripheral pigment, (vi) schizonts produced 8-12 merozoites, (vii) gametocytes measured 6-7 µm in diameter and were numerous late in the infection in mice, and (viii) the pigment was yellow.

An additional similarity is that the parasitaemia in Bruce-Chwatt and Gibson's subinoculated mouse fell after a week and there was then a spontaneous recovery. A similar course of infection is often seen in mice inoculated with *P.v. brucechwatti* from *Thamnomys*. One difference is the vacuolation of macrogametocytes of Bruce-Chwatt and Gibson's parasite noted by Garhnam (1966). This may, however, be an indication of senility.

It is concluded that the parasites are the same, and that the strain isolated by Bruce-Chwatt and Gibson by inoculating a mouse with blood pooled from several rodents identified as *Praomys tullbergi* originated from an incorrectly identified specimen of *Thamnomys rutilans*. This is supported by the results of the examination of 2,617 rodents of 9 different species, in Western State, Nigeria, by the inoculation of blood into white mice. The sample included 1,607 specimens of *Praomys tullbergi*. With the exception of Bruce-Chwatt and Gibson's rodent, malaria parasites were isolated only from thicket rats, *Thamnomys rutilans*, six out of 18 of which were infected (Killick-Kendrick, 1973 *a*).

Eight species of malaria parasites are described from African rodents (Killick-Kendrick, 1974*a*; Carter and Walliker, 1975). Four, which are from hosts other than murine rodents, are clearly different from the Nigerian parasite. Three of these, from *Anomalurus* spp., form a distinctive group readily differentiated from the parasites of murine rodents by the morphology of the blood stages (Killick-Kendrick, 1973 *b*). Another, *P. atheruri* van den Berghe, Peel, Chardome and Lambrecht, 1958 of *Atherurus africanus*, differs from the Nigerian parasite by, among other characters, the small number of merozoites produced by blood schizonts (Garnham, 1966) and the low infectivity to mice (Landau, Adam and Boulard, 1969).

The remaining four species are from murine rodents. They are principally or solely parasites of thicket rats of the Lower Guinea Forest. They fall into two groups namely (i) the *berghei* group consisting of *P. berghei* Vincke and Lips, 1948 and *P. yoelii* Landau and Killick-Kendrick, 1966 (Killick-Kendrick, 1974 *b*) and (ii) the *vinckei* group consisting of *P. vinckei* Rodhain, 1952 and *P. chabaudi* Landau, 1965 (Carter and Walliker, 1975).

From the morphology of the blood stages, the Nigerian parasite is indistinguishable from *P. vinckei*. Within this species, three subspecies have been named viz. *P.v. vinckei* Rodhain, 1952 from Katanga, *P.v. lentum*, Landau, Michel, Adam and Boulard, 1970 from Brazzaville, and *P.v. petteri*, Carter and Walliker, 1975 from the Central African Republic (CAR). This classification gives taxonomic recognition to populations which are thought now to have a discontinuous distribution; it recognises their close relationships and probably reflects the present point of evolution within the species.

The distribution of *P.v. brucechwatti* is assumed to be restricted to Nigeria. Between the type locality and other places in which *P. vinckei* is found there are four faunal barriers of differing importance (Killick-Kendrick, 1973 *a*). On these zoogeographical grounds *P. vinckei* from Nigeria cannot confidently be assigned to one of the named subspecies, and it is therefore given a new subspecific name.

There are minor differences in the sporogonic stages of the subspecies of *P. vinckei* (*Table II*). The oocyst of subspecies *vinckei* is smaller than that of the others, and the sporozoites are significantly shorter. The sporozoites of *P.v. lentum* are unusually long (the longest of the genus). Morphological differences between the sporogonic stages of the subspecies *lentum* and *brucechwatti* are slight and of

uncertain significance. I believe the comparatively large standard deviations in the measurements of the sporozoites of all subspecies except *P.v. vinckei* are associated with poor infectivity, and that great weight cannot be placed on small differences.

TABLE II. — Sizes of sporogonic stages of *P. vinckei* ssp. in *Anopheles stephensi*

	(1) *P.v. vinckei*	*P.v. lentum*	(3) *P.v. petteri*	(1) *P.v. brucechwatti*
Oocysts (μm)	40	47 (2)	50	54
Sporozoites:				
(i) lengh (μm)	12.3	19.5	16.6	14.7
(ii) ± SD (μm)	0.99	2.64 (1)	2.25	3.04

(1) Original.
(2) Landau *et al.* (1970).
(3) Carter & Walliker (1975); sporozoites from rupturing oocysts.

Preliminary observations on the enzymes of the *vinckei* group were given by Carter (1973) and Carter and Walliker (1975). Refinements of techniques have led to a more precise analysis (Carter, 1974), summarized in *Table III*. All subspecies of *P. vinckei* share the type GDH-6, one of the characters separating *P. vinckei* and *P. chabaudi*. Within the species *vinckei, P.v. brucechwatti* shares the forms GPI-6 and LDH-9 with *P.v. lentum* and 6 PGD-6 with *P.v. vinckei*. Carter's observations strongly confirm the separate specific status of *P. vinckei* and *P. chabaudi* and the close relationships between susbpecies of *P. vinckei*.

In spite of the low infectivity of sporozoites of *P.v. brucechwatti* in *A. stephensi*, Bafort (1971) was able to infect a mouse and demonstrate exoerythrocytic schizonts of strain 1/69. At 61 hrs after the inoculation of sporozoites one apparently mature schizont measured 48 × 38 μm. Blood taken from the mouse at 65 hrs was infective to other mice; the maturation time of the tissue stages in the liver of the mouse was therefore between 61 and 65 hrs.

Diagnosis.

Plasmodium vinckei brucechwatti subsp. nov.

Synonym: *P. vinckei chwatti* Garnham, 1973 (a *nomen nudum*).

Blood stages. No predilection for immature erythrocytes; no enlargement or stippling of the host-cell; infected cells are progressively distorted as parasites grow; ring forms commonly have double nuclei; an accessory dot is occasionally present; young trophozoites are vacuolated; old trophozoites are compact, not amoeboid; at the first division of the nucleus the diameter of schizonts is at least half that of the erythrocyte; nuclei of schizonts are of irregular shape with one deeply stained part; schizonts almost fill the host-cell and produce 12 (8-14) merozoites in mice. 8-12 in naturally infected thicket rats; gametocytes are dimorphic, fill the host-cell and measure 6-7 μm in diameter; their nuclei are always peripheral; pigment is yellow;

it appears early, is mainly at the periphery and, in schizonts, it clumps into a mass before merozoites are fully formed; Carter's designations of this forms of enzymes of strains N 48 and 1/69 are: GPI-6, 6 PGD-6, LDH-9 and GDH-6.

TABLE III. — Forms of isoenzymes of blood stages of malaria parasites of the *vinckei* group (Carter, 1974)

	P.v. vinckei (2 isolates) Katanga	*P.v. lentum* (4 isolates) Brazzaville	*P.v. petteri* (4 isolates) CAR	*P.v. brucechwatti* (2 isolates) Nigeria	*P. chabaudi* (22 isolates) CAR	*P. chabaudi* ssp. (2 isolates) Brazzaville
GPI	7	6,11	5,9	6	4	8
6 PGD	6	5	5	6	2,3,7	2
LDH	6	7,9	7	9	2,3,4,5	8,10
GDH	6	6	6	6	5	5

Sporogonic stages. At 25 °C sporozoites enter the salivary glands mainly on day 13; sporozoites from *A. quadrimaculatus* and *A.l. atroparvus* are not infective and from *A. stephensi* rarely so; pigment in 3-day oocysts is abundant and lies in short lines, sometimes fan-shaped; the diameter of mature oocysts is typically 54 μm; sporozoites in stained smears of infected salivary glands measure 14.7 μm (SE 0.5 μm).

Exoerythrocytic stages (Bafort, 1970). In the liver of white mice, mature forms at 61 hrs have a mean diameter of 43 μm; merozoites invade the blood 61-65 hrs after the inoculation of sporozoites.

Hosts. Thamnomys rutilans (Peters, 1876) is the type host; mice and multimammate rats (*Praomys natalensis*) are susceptible; natural vector(s) unknown; *A. gambiae* B is insusceptible; *A. stephensi, A. quadrimaculatus* and *A.l. atroparvus* are unsuitable laboratory hosts.

Geographical distribution. Believed to be restricted to Western Nigeria; the type locality is 100 m above sea level in secondary forest and farmland on the edge of Ilaro Forest Reserve near the village of Ilobi, Western State, Republic of Nigeria (longitude 3° 03′ E, latitude 6° 45′ N).

Type material in the writer's collection will be deposited in the Wellcome Museum, London; strains N48 and 1/69 are maintained in the Protozoan Genetics Unit, Institute of Animal Genetics, Edinburgh, Scotland; strain N 48 is here designated the type strain.

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