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Further description of blood stages of *Plasmodium petersi* from *Cercocebus albigena* monkey

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Abstract. Additional information is given on the erythrocytic stages of *Plasmodium petersi* (Poirriez, Baccam, Dei-Cas, Brogan et Landau, 1933), which was found in a *Cercocebus albigena* monkey from the Central-African Republic. The first colour pictures of *P. petersi* are presented. In 60% of young trophozoites, the vacuole is divided into two or three parts by thin cytoplasmic streaks. In young trophozoites and almost mature schizonts, 80% of nuclei are oval or kidney-shaped; they are two-coloured; measurement of their surface area shows that it is about twice that of the nuclei of *P. gonderi* at the same stages. Studies using polarised light show that most of the pigment granules are elongated (spindle-shaped) and found at the periphery of old trophozoites and schizonts. *P. petersi* can easily be distinguished from *P. gonderi* and *P. georgesi*, the two other species found so far in *Cercocebus* monkeys, which are regarded as the African equivalents of the Asian macaques.

Until recently, *Plasmodium gonderi* (Sinton et Mulligan, 1933) had been believed to be the sole species of the family Plasmodiidae infecting monkeys of Central Africa (Collins 1988). In 1993, we described two new species of the genus *Plasmodium* in a *Cercocebus albigena* monkey which was caught in the south of the Central-African Republic: *P. petersi* and *P. georgesi* (Poirriez et al. 1993).

An additional description of the erythrocytic forms of *P. petersi* is given herein, including quantitative analysis of life-cycle stages, additional measurements, data on the pigment obtained by using polarised light, as well as first colour pictures of the blood stages of this recently discovered species.

MATERIALS AND METHODS

Plasmodium petersi was described and further studied in the first four Giemsa-coloured blood smears which were made in Bangui on the 17th and 20th of April 1988, from the *Cercocebus albigena* monkey (Poirriez et al. 1993). All the parasites seen were counted and measured on the microscope with a micrometric ocular scale. The size of each parasite stage, its nucleus and its host red blood cell was determined.

The parasitaemia was counted in 500 microscopic fields (taken from two slides), at a magnification of x 625 (with immersion oil), with each microscopic field containing about 1000 red blood cells.

Colour photographs were taken with a Zeiss Axiophot photo-microscope, at a magnification of x 1000; measurements were taken again from enlarged photographs. Surface areas of parasite nuclei were calculated by projection of colour slides (including scale bars) on a sheet of squared paper; 30 nuclei of each stage were measured.

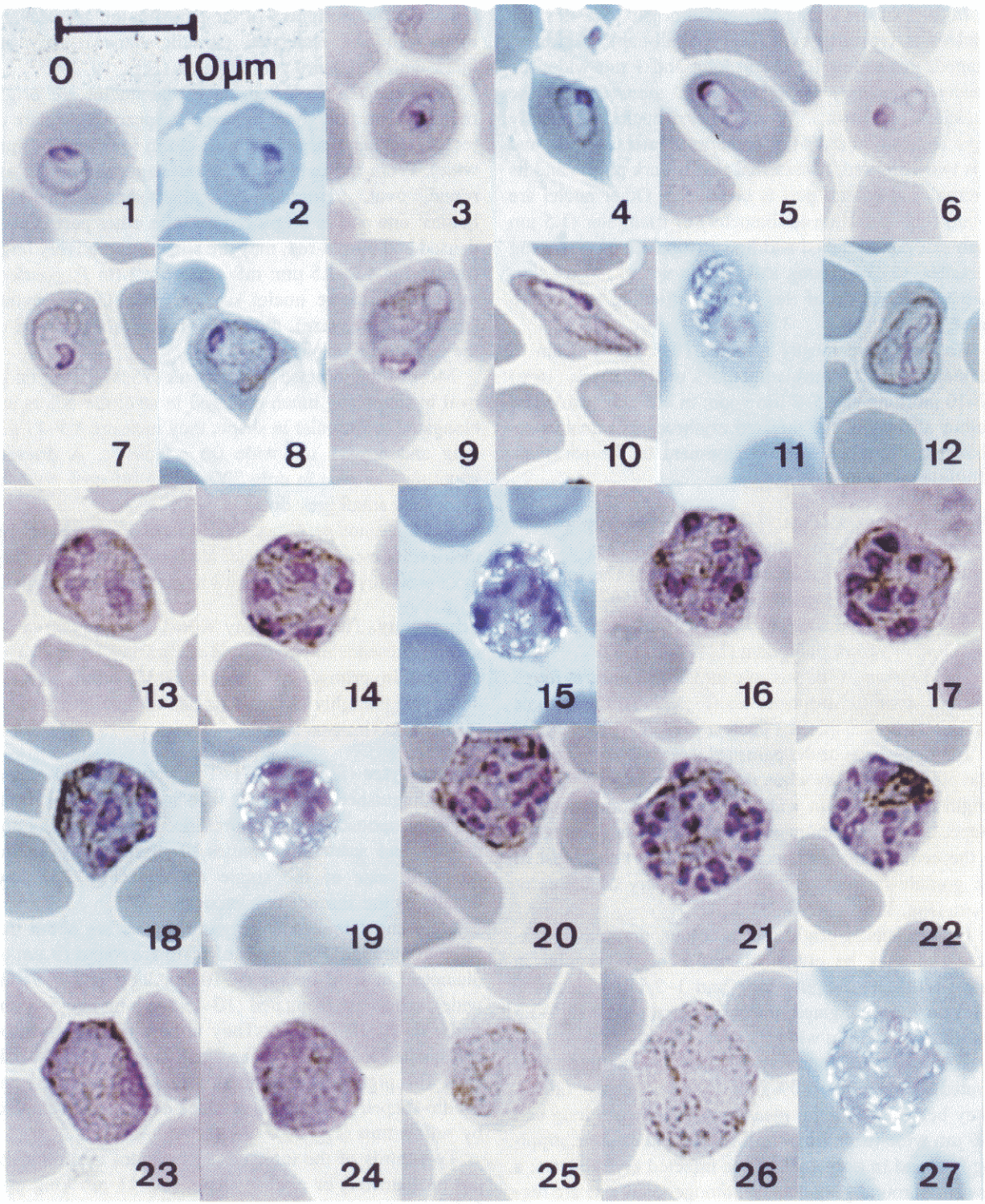
The morphology and distribution of the pigment granules were studied by polarised light (Field et al. 1956, Peters et al. 1976).

RESULTS

Although only a few parasites were found in these smears, the identification of *Plasmodium petersi* was facilitated because, at that time, *P. gonderi* was present in limited numbers.

Parasitaemia. In the smears made on the 17/4/88, we counted 470 parasites per mm^3 (0.009%): 270 trophozoites, 130 schizonts and 40 gametocytes of *P. petersi*; 20 trophozoites and 10 schizonts of *P. gonderi*. In the smears made on the 20/4/88, we counted 590 parasites per mm^3 (0.01%): 440 trophozoites and 120 gametocytes of *P. petersi*; 30 trophozoites of *P. gonderi*.

Young trophozoites (Figs. 1–6). Most of them (80%) have an oval shape: 2.5–5 μm long and 2–3 μm wide, mean (m) = 3.7/2.6 μm; the others are round bodies (3 μm in diameter). The pale blue cytoplasm appears as a narrow strip around the vacuole and, in 60% of young trophozoites, this vacuole is divided into two (50%) or



Figs. 1–27. *Plasmodium petersi*; Giemsa coloured blood smears from a *Cercocebus albigena* monkey caught in the Central-African Republic; **1–6** – young trophozoites; **7–12** – late trophozoites; **13–22** – schizonts; **23–24** – macrogametocytes; **25** – young microgametocyte; **26–27** – microgametocytes; **11, 15, 19, 27** – shape and distribution of the pigment granules, as viewed by polarized light.

three (10%) parts by thin cytoplasmic streaks issued from the peripheric cytoplasmic circular belt.

In 80% of the young intra-erythrocytic parasites, the nucleus is typically oval (like a skull-cap) or kidney-shaped, measuring 1.5–2 µm long and 1 µm wide; the mean surface (mS) = 0.99 µm² (in *P. gonderi*, mS of the nucleus = 0.43 µm²). The *P. petersi* nucleus is mostly polar in position (80%) or sometimes lateral (10%), and it is two-coloured: its external part is dark purple and its internal and central part is bright red. Other nuclei are round (about 1.2 µm in diameter) or triangular (1.5 µm each side) or stretched and linear (for 12% of the young parasites) (2–3 µm long and 0.5 µm wide), mostly situated in the middle of the vacuole, dividing it into two parts.

Host cells are mostly rounded (84%) (6.5–8 µm in diameter; m = 7.3 µm), sometimes oval in shape (16%) (7–10 µm long and 5–7 µm wide; m = 8.4/5.9 µm). The colour affinity of the infected erythrocytes remains unchanged throughout the development of the parasites. Multiple infections were not seen.

Old trophozoites (Figs. 7–12). The late trophozoites are never amoeboid, always compact. They occupy about two-thirds of the host erythrocyte. The majority of late trophozoites are oval in shape (75%) (5–9 µm long and 2.5–7 µm wide; m = 6.5/4.5 µm) and the others are round (13%) or polygonal (12%).

The division of the vacuole into 2 or 3 parts is found again in some old trophozoites (Figs. 7–9); the vacuole disappears before the first nuclear division (Fig. 12).

The cytoplasm of the parasites is light-blue in colour. The pigment appears when the parasites exceed 5 µm in length and 2.5 µm in width. Some grains are round in shape, others are elongated, bacilliform; they are black in the centre and have peripheral yellow tints. Most of the granules are situated at the periphery of the cytoplasm (Fig. 11).

The nucleus is bright red in colour, granular in texture, and may be either triangular or rectangular in shape (80%) (1.5–3 µm long and 1–2 µm wide; m = 2/1.6 µm), or elongated, linear in shape (20%) (2.5–3.5 µm long and 0.5 µm wide).

Host cells are either oval (40%) (sometimes fimbriated), polygonal (40%), or round in shape (20%). They begin to enlarge, measuring 7.5–11 µm long and 5–9 µm wide (m = 9/6.5 µm). A fine and discrete stippling is found in only 25% of the infected erythrocytes: a few small round grey dots of homogeneous size are regularly dispersed. They appear to be numerous in only a few cases.

Schizonts (Figs. 13–22). They are round or oval bodies; from the six-nucleated stage, they almost fill the host cell, measuring 8–11 µm long and 6–11 µm wide (m = 9.2/8 µm).

The cytoplasm of the parasites is pale blue in colour. In the immature schizonts, about half of the pigment remains at the periphery of the parasite; the granules are often thin and elongated (spindle-shaped); they are black with peripheral yellow flecks (Figs. 15, 19).

Until the ten-nucleated stage, the nuclei are bright red in colour, polygonal in shape (trapezoidal, square or rectangular) and large in size (2–3 µm long and 1–2 µm wide). From the twelve-nucleated stage, the nuclei are round, oval, triangular or kidney-shaped two-tone bodies: one part is bright red and the other part is condensed and purple-red; they are still comparatively large (measuring 1.2–1.5 µm; mS = 0.9 µm²) (in *P. gonderi*, mS of the mature nuclei = 0.38 µm²). On all points (shape, texture, size), they are similar to the nuclei of the young trophozoites.

Most of the infected erythrocytes (75%) are round or oval in shape and much enlarged in size; the others are elongated or irregular in shape; they measure 8.5–11 µm long and 6.5–11 µm wide (m = 9.5/8.5). A discrete stippling was seen in only 20% of the infected erythrocytes (few small grey dots).

We have not seen any totally mature schizonts, but the development of the nuclei leads us to think that they most probably contain 12 to 18 large bicoloured nuclei.

Schizogony. The schizogony seems very synchronous, and the presence of two broods of parasites (as it is most frequent in primate malarias) makes us think that a 48h-cycle is highly likely. This has to be confirmed by further experimental studies.

Gametocytes (Figs. 23–27). The young sexual stages are distinguished, sometimes with some difficulty, from the late trophozoites by the presence of increasingly numerous and scattered granules of pigment, by an increase in size of the single nucleus (Fig. 25), and usually by the absence of a vacuole.

Male and female mature gametocytes are about the same in number. Two thirds of them are round (9 µm in diameter) or oval bodies (9–10 µm long and 6–9 µm wide); others are elongated (10–13 µm long and 5–8 µm wide; m = 10/7.5 µm). They almost fill the erythrocytes, often leaving only one small side of the host cell free. The pigment is scattered, made of long and fine, needle-shaped granules; their black centre is surrounded by yellow tints (Fig. 27).

Two-thirds of the infected erythrocytes are round (9 µm in diameter) or oval in shape (10–11 µm long and 7–9 µm wide); the others are distorted, a few are polygonal or fimbriated (10–13 µm long and 5.5–8.5 µm wide). The stippling is not constant, and is found in only 50 % of the cells infected with gametocytes; it is very light, made of a few fine grey dots.

The cytoplasm of the macrogametocyte stains a bright blue colour; the bright red and granular small

nucleus is most often lateral, like a small segment of a sphere (measuring 2.5–4 µm long and 1–1.5 µm wide) (Figs. 23, 24).

The nucleus of the microgametocyte is large, often ill defined, diffused, not well demarcated from the blue grey cytoplasm (Fig. 26). Nuclear limits are more easily observed when seen under polarised light (Fig. 27). The nucleus occupies about one-third of the parasite, but it may be smaller and somewhat triangular (3.5 µm each side), especially in younger microgametocytes.

Gametocytes are of the short-living type: they were not found in the peripheral blood five days later.

DISCUSSION

The malaria parasites infecting monkeys of the genus *Cercocebus* in Central Africa are more various than it was thought until 1993. The successive or concurrent discovery of two or three species of *Plasmodium* in the same monkey host is now a well known phenomenon. At a certain time, one species of parasite may predominate in the peripheral blood to the almost complete exclusion of the other (Sinton 1934); the other species will be discovered only if the investigations are repeated. It was the case for the lemurs in Madagascar; before 1980, only three species of *Plasmodium* had been described (only seven animals were studied); from 1982 to 1988, extensive transversal (made on 171 lemurs) and longitudinal (3 lemurs were followed during 30 to 70 days) studies have led to the description of four new malarial species (Landau et al. 1989). There are numerous other examples in the literature showing that careful extensive studies of some Asian apes and monkeys have led to the discovery of new species in the genus *Plasmodium* (Eyles 1963, Dissanaike et al. 1965, Peters et al. 1976).

The size of the nuclei of young trophozoites and of merogonic forms, as well as the size of merozoites, is sometimes a helpful criterion for the distinction between two closely-related species of malaria parasites (for example between *P. vivax* and *P. ovale*) (Bray 1957). Our observations show that the measurement of the nucleus surface may be much more discriminating.

P. petersi can easily be distinguished from *P. gonderi*, which shows: an intense and peculiar stippling, an increasing pallor of the infected erythrocytes, little or any enlargement of the host cells (mostly less than 8.5 µm, except in case of multiple infections), a smaller nucleus of the merozoite and of the young trophozoite (this nucleus is mostly dense, homogeneous and round, measuring less than 1 µm in diameter), and a more compact black pigment (in short rods) (Rodhain and Van Den Berghe 1936, Garnham 1966, Coatney et al. 1971).

P. petersi may also be easily distinguished from *P. georgesi*, which shows: an intense stippling with numerous purple dots of unequal size, a frequent polygonal appearance of the much enlarged infected erythrocytes, a crescent shape of the nucleus of the young trophozoite, a fine round yellow pigment, and the presence of 22 to 26 nuclei in the almost mature schizonts (Poirriez et al. 1993).

There is much more to learn about the genus *Plasmodium* in African monkeys, especially in those of the genus *Cercocebus*, which are regarded as the African equivalents of the Asian macaques (Dandelot 1971).

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