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PLASMODIUM BRYGOOI SP. N. FROM *CHAMAELEO BREVICORNIS* OF MADAGASCAR, WITH A REDESCRIPTION OF *PLASMODIUM ROBINSONI* (BRYGOO, 1962) FROM ITS TYPE HOST AND AN ADDITIONAL HOST, *CHAMAELEO PARSONI CRUCIFER*

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SUMMARY. *Plasmodium brygooi* sp. n., described from *Chamaeleo brevicornis* of Madagascar, has schizonts smaller than or equal to host cell nucleus size. Schizonts produce 10-16 merozoites usually arranged peripherally around clusters of dark pigment granules. Gametocytes, oval or elongate, average slightly larger than host cell nuclei. Schizonts and gametocytes usually lie polar in enlarged erythrocytes. Hypertrophy of host cell nuclei is produced by schizonts but not by gametocytes. *Plasmodium robinsoni* (Brygoo) 1962 is a much larger parasite of *C. brevicornis* and *Chamaeleo parsoni crucifer*. Schizonts are round, oval or elongate, and approximate gametocytes in size. The 40-74 merozoites fill schizonts which usually contain a single, central large mass of golden pigment. Gametocytes are oval to elongate or bulky, with macrogametocytes larger and less elongate than microgametocytes. Both schizonts and gametocytes average three times or more the size of host cell nuclei. Host cells of asexual or sexual stages show hypertrophy but only erythrocytes parasitized by schizonts have enlarged nuclei.

Key-words: Systematic. *Plasmodium brygooi* n. sp. *P. robinsoni*. *Chamaeleo*. Madagascar.

Plasmodium brygooi n. sp. parasite de *Chamaeleo brevicornis* à Madagascar, avec la redescription de *Plasmodium robinsoni* (Brygoo, 1962) chez son hôte type et chez un deuxième hôte, *Chamaeleo parsoni crucifer*.

RÉSUMÉ. Description de *Plasmodium brygooi* n. sp. chez *Chamaeleo brevicornis* de Madagascar et redescription de *Plasmodium robinsoni* (Brygoo), 1962 chez *C. brevicornis* et *C. p. crucifer*. Les deux espèces se différencient par leur taille, *P. robinsoni* étant beaucoup plus grand que *P. brygooi*.

Mots-clés : Systématique. *Plasmodium brygooi* n. sp. *P. robinsoni*. *Chamaeleo*. Madagascar.

During his exhaustive studies of the parasites of Madagascan chamaeleons from 1954 to 1962, Edouard-R. Brygoo described *Haemamoeba robinsoni* in *Chamaeleo brevicornis* from Fiherenana, Sous-Préfecture de Moramanga. He sub-

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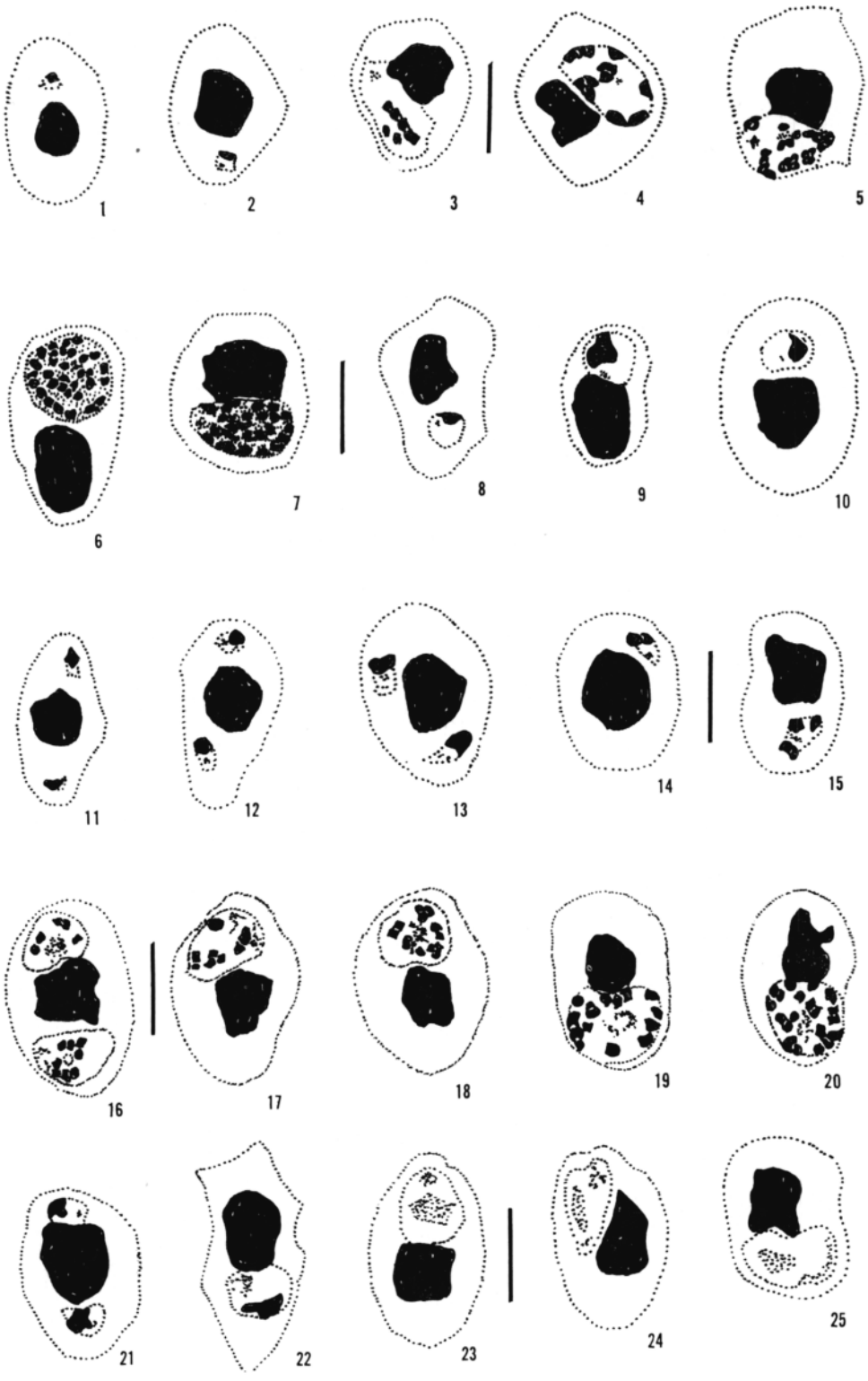


FIGS. 1-25.

sequently reported its presence in the same host from another locality on the east coast, Perinet (Brygoo, 1963). There have been no further reports of this or any other saurian malarial parasite from Madagascar.

As part of an undertaking to re-describe the known species of *Plasmodium* parasites of reptiles, the type slide of *Haemamoeba (= Plasmodium) robinsoni* and a slide from another *C. brevicornis* collected in 1972 were examined. Although a few gametocytes of *P. robinsoni* were seen on the latter slide, most parasites found represent an undescribed *Plasmodium* species. Study of a *Plasmodium* infection in another host species, *Chamaeleo parsoni crucifer*, collected in Perinet, Madagascar in 1969, revealed a parasite which cannot be distinguished from *P. robinsoni*. With this new material at hand it is appropriate to re-describe *P. robinsoni* from both the type and additional host, and to provide taxonomic designation of the second species present.

Materials and methods

The slides examined had been fixed in absolute methanol and stained by Giemsa 13-24 years earlier. Preservation of the type slide of *P. robinsoni* (1961) was reasonable and that of the slide from *C. parsoni* (1969) excellent, but the second slide from *C. brevicornis* (1972), containing the undescribed species, had faded and required restaining. Vestiges of stain remaining were removed by immersion in acetone, followed by absolute methanol. It was then placed for 5 minutes in absolute ethanol which contained about 5 drops of acetic acid. The slide was restained in dilute Giemsa solution, 0.5 ml stain in 10 ml distilled water at pH 7.0 for 6 hours. After rinsing in distilled water, excess stain was removed by immersion in acetone with frequent checking at 400 × magnification. When differentiation appeared to be the best obtainable, the slide was air-dried and mounted in Euparol vert. Slides were examined, parasites measured by calibrated ocular micrometer, and photographed at 1,000 × under oil immersion. Statistical analysis was done using the Microstat package (Ecosoft, Inc.) for IBM personal computers. Taxonomic characters employed below are those of Telford (1974, 1979). The hepantotype slides are deposited at the Museum d'Histoire naturelle, Paris.

FIGS. 1-25. — *Plasmodium robinsoni* from *Chamaeleo brevicornis* (1-10) and *C. parsoni crucifer* (11-25).

1,2, 11-13: trophozoites.

3-5, 14-20: schizonts.

6-7: nearly mature schizonts.

8-10, 21-25: young gametocytes.

All host cells erythrocytes. Vertical bars represent 10 μm.

Results

TAXONOMIC REDESCRIPTION

Plasmodium robinsoni (Brygoo), 1962 *(figs. 1-19).*

Description from type host, *Chamaeleo brevicornis (figs. 1-10, 44-49):* Type infection chronic, comprised almost entirely of gametocytes at parasitemia of < 0.1 %. Trophozoites *(figs. 1, 2)* 2 × 1.5-4 × 3.5 μm, triangular, nearly square or oblong, with smallest pigmented. Most developing schizonts *(figs. 3-7)* oblong in form, 9-12 × 6-7 μm, with 6-26 nuclei. An apparently mature, nearly round schizont, 10 × 9 μm, with 47 nuclei was polar in the host cell (HC). Immature gametocytes with prominent nuclei *(figs. 8-10)*, rounded or broadly lentiform, 5-6 × 4-6, occupied polar positions in HC. Mature gametocytes *(figs. 44-49)* 12-18 × 5-12 μm ($\bar{x}$ 14.98 ± 0.2 × 7.44 ± 0.3, N = 50), with mean LW 110.52 ± 3.5 μm² (75-170). Gametocytes usually elongate, with mean L/W ratio 2.14 ± 0.08 (1.1-3.4), and ratio LW: HC nuclei 3.07 ± 0.14 (2.1-5.2). Macrogametocytes *(figs. 44-47)* larger (LW 113.4 ± 3.9 μm², N = 42) than microgametocytes (95.4 ± 3.2 μm², N = 8, *figs. 48, 49*), usually less elongate (L/W 2.07 ± 0.08 *vs.* 2.49 ± 0.24).

Description from *Chamaeleo parsoni crucifer (figs. 11-43):* Trophozoites *(figs. 11-13)* 2 × 1-4 × 3 μm, elongate or oblong, with smallest unpigmented. Occasional trophozoites had a short cytoplasmic projection at one end. Smallest binucleate schizont *(fig. 14)* 4 × 2 μm; the second nuclear division occurred when size had doubled, to 5.5 × 3 μm. With later nuclear divisions *(figs. 15-20, 26-29)* schizonts became lentiform, more rounded, or elongate, with pigment granules often clustered centrally, surrounded by nuclei. Mature schizonts and segmenters *(figs. 30-33)* 16.1 ± 0.9 × 8.8 ± 0.3 μm (11-23 × 7-11, N = 17), with 40-74 merozoites ($\bar{x}$ 56.7 ± 2.9). Schizont LW 99-184 μm² (x 138.8 ± 6.1), with ratio LW: HC nucleus size 3.1 ± 0.2 (2.0-4.3, N = 16). Schizonts usually round or oval *(fig. 30)* with large, light golden pigment mass often centered amidst nuclei. Some elongated schizonts *(figs. 31-33)* occasionally divided into two portions by HC nucleus, only one portion containing pigment. Smallest apparent gametocytes *(fig. 21)* 4-6 by

FIGS. 26-49. — *Plasmodim robinsoni* from *C. parsoni crucifer* (26-43) and *C. brevicornis* (44-49, type infection).

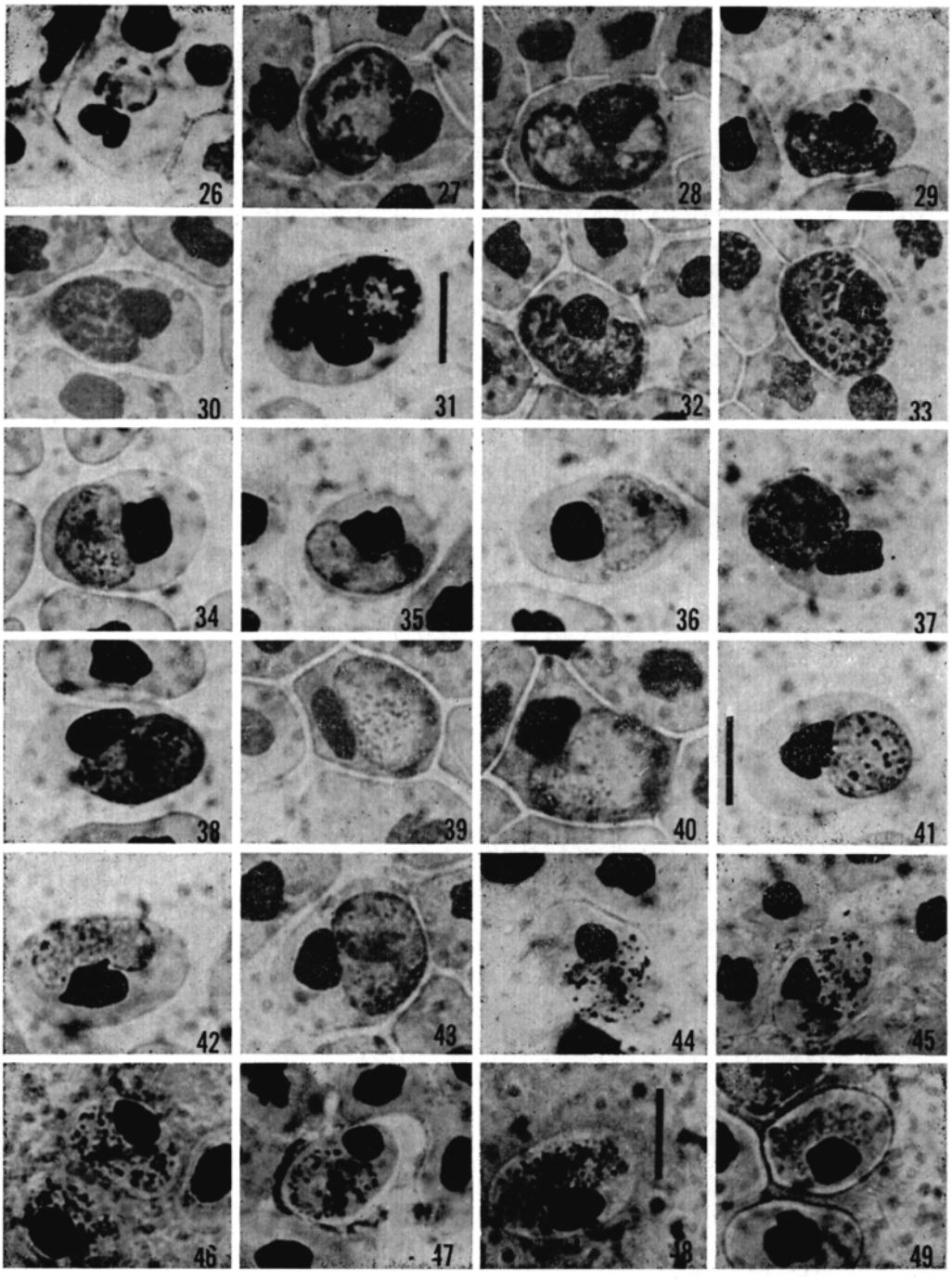
26-29: schizonts.

30-33: mature schizonts and segmenters.

34-40, 44-47: macrogametocytes.

41-43, 48, 49: microgametocytes.

All host cells erythrocytes except for figure 26 which may be a proerythrocyte. Vertical bars represent 10 μm.



FIGS. 26-49.

2-3 μm, with large, often central nuclei. Immature gametocytes *(figs. 22-25)* round, oval, lentiform or, rarely, elongate with prominent clusters of dark pigment granules. Mature gametocytes *(figs. 34-43)* 13.9 ± 0.3 × 9.4 ± 0.2 μm (9-20 × 6-13, N = 70), with mean LW 130.2 ± 3.9 μm² (72-221). Gametocytes usually oval, with mean L/W 1.54 ± 0.05 (1.0-2.8); average ratio LW: HC nucleus size 3.12 ± 0.16 (1.6-5.0). Macrogametocytes *(figs. 34-40)* larger in LW (133.2 ± 5.1 μm², N = 45) than microgametocytes (124.8 ± 6.0 μm², N = 25). Microgametocytes *(figs. 41-43)* usually more elongate (L/W 1.78 ± 0.08) than macrogametocytes (1.40 ± 0.06). In both hosts, cells infected with schizonts or gametocytes enlarged and distorted, with nuclei always displaced and often distorted; only schizonts produced nuclear hypertrophy. Schizonts and gametocytes most commonly polar or lateropolar in *C. parsoni* erythrocytes, gametocytes more often lateral or lateropolar in *C. brevicornis*.

DIAGNOSIS: A *Plasmodium* parasite of Madagascan *Chamaeleo* species which is characterized by its large round, oval or elongate schizonts approximately equal to the gametocytes in size. Schizonts produce 40-74 merozoites. Both schizonts and gametocytes average three times or more the size of host cell nuclei. Macrogametocytes are larger than microgametocytes, on average, but are less elongate. Host cells of asexual and sexual stages show significant hypertrophy; only schizonts cause enlargement of host cell nuclei.

TYPE LOCALITY: Fiherenana, Moramanga Sub-Prefecture, Madagascar (48° 24'E, 18° 28' S).

TYPE HOST: *Chamaeleo brevicornis*, Gunther, 1879.

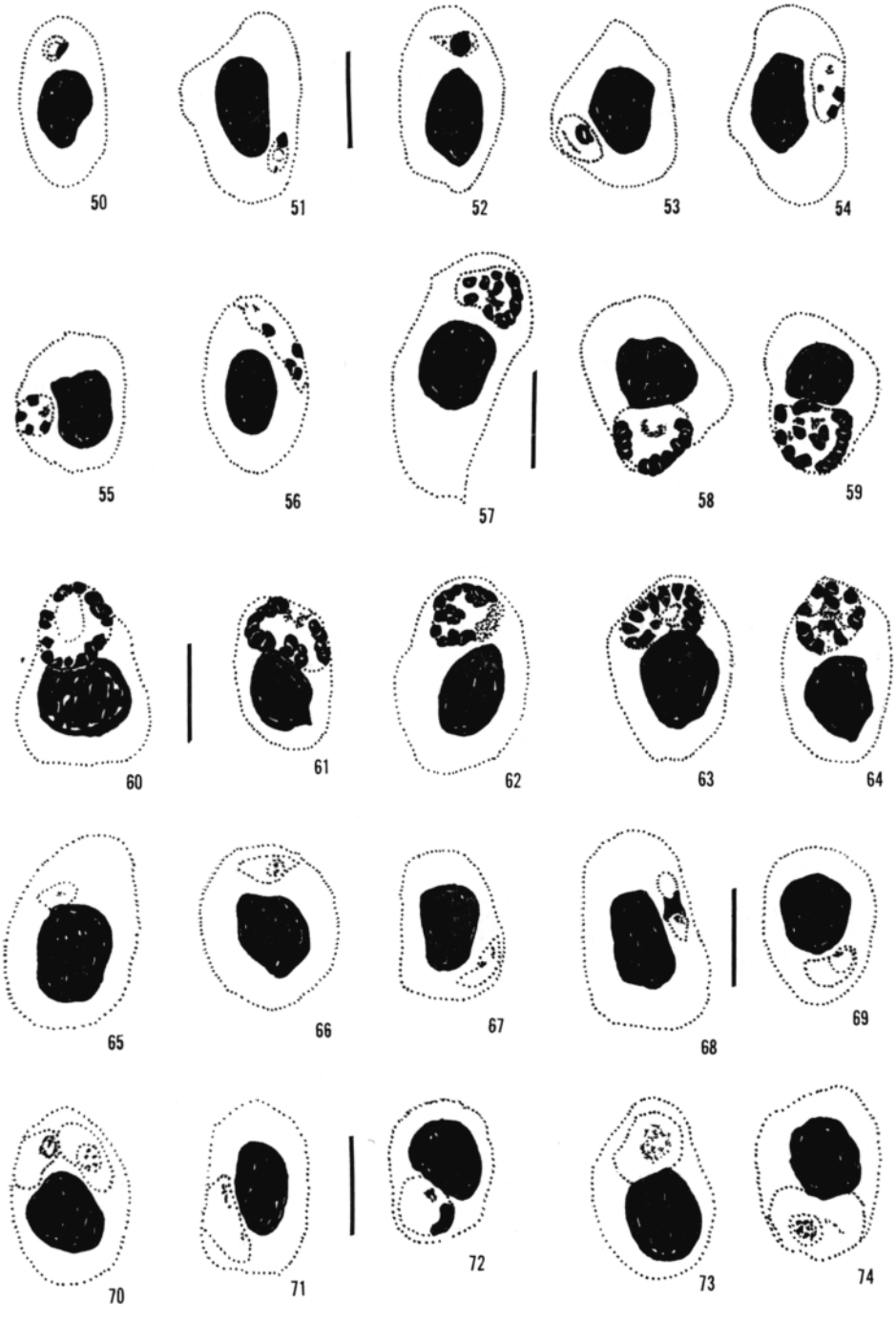
ADDITIONAL HOSTS: *Chamaeleo parsoni crucifer.*

GEOGRAPHIC RANGE: Known only from Fiherenana and Perinet, Madagascar.

TAXONOMIC DESCRIPTION

In recognition of his outstanding contribution to the parasitology of Madagascan chamaeleons and other saurians, the second parasite found in *Chamaeleo brevicornis* is designated:

FIGS. 50-74. — *Plasmodium brygooi* n. sp. from *C. brevicornis*.
50-52: trophozoites.
53-58: schizonts.
59-64: mature schizonts.
65-74: young gametocytes.
Host cell 50 is a proerythrocyte, remainder erythrocytes. Vertical bars represent 10 μm.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Figs. 50-74.

Plasmodium brygooi sp. n. *(figs. 50-90).*

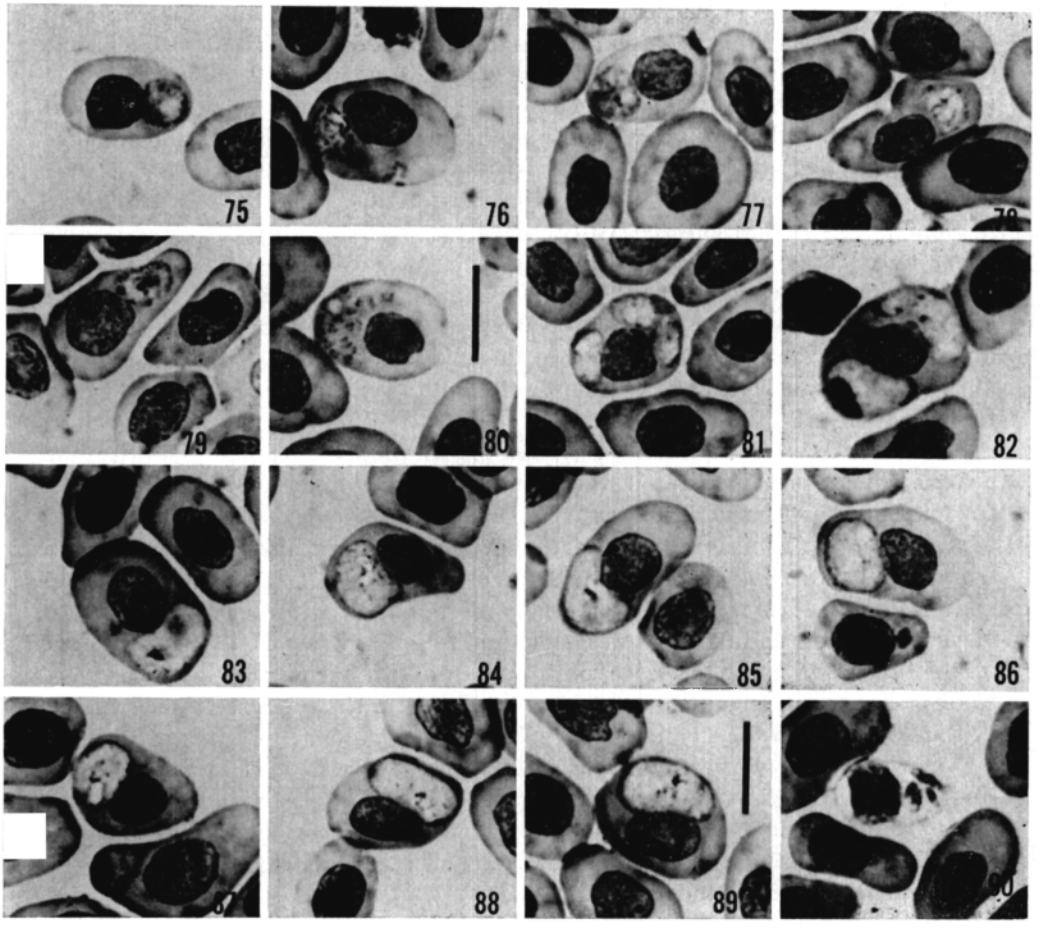
DESCRIPTION: Trophozoites *(figs. 50-52)*: Smallest parasites 2.5-4 × 1.5-2 μm, oval, with prominent vacuole located centrally to terminal nucleus. Some trophozoites 4 × 2 μm had a single dot of dark pigment. Binucleate schizonts *(figs. 53-54)* 7-8 × 3.5-4 μm, elongate, without vacuoles. Size at second nuclear division variable: tetranucleate schizonts *(figs. 55, 56)* 4 × 4-12 × 3 μm, round to elongate. Schizonts with 10-16 nuclei *(figs. 57-64, 75-79)*, oval, oblong or lentiform, with nuclei usually arranged peripherally around several large clusters of dark pigment. Segmentation appeared imminent in those with 12-16 nuclei *(figs. 59-64)*. Mature schizonts *(figs. 59-64, 79, 80)* 7.9 ± 0.2 × 6.3 ± 0.2 μm (6-9 × 5-8, N = 16), with mean LW 49.0 ± 1.7 μm² (36-64), and 10-16 merozoites ($\bar{x}$ 13.8 ± 0.5, N = 17). Most formed as rosettes (81 %), were oval (13 %) or lentiform (6 %). Pigment granules rarely coalesced into single, golden mass, usually as 3 or 4 prominent dark clusters. Small, elongated parasites 3.5-7 × 1.5-2.5 μm *(fig. 65)*, with neither nucleus nor vacuole probably young gametocytes. Most had 1-2 small dots of dark pigment. At 5-7 × 2-3 μm large, single nuclei sometimes visible. As gametocytes grew *(figs. 66-74, 81)*, pigment granules usually formed prominent cluster, often clearly within vacuole *(figs. 66, 69, 70, 74)*. Mature gametocytes *(figs. 82-89)* 11.5 ± 0.2 × 8.1 ± 0.2 μm (9-15 × 5-10, N = 48), with mean LW 92.5 ± 2.3 μm², and shape round to elongate, with mean L/W ratio 1.47 ± 0.05 (1.1-3.0). Ratio of LW: HC nuclei 1.74 ± 0.07 (1.0-3.4). Gametocyte sex difficult to determine due to restaining, but no apparent difference in size or shape between those which could be identified. Occasional young schizonts in immature cells; all mature schizonts and gametocytes occupied polar or lateropolar positions in erythrocytes, causing hypertrophy and distortion of the cell, nuclear displacement and often distortion, and with schizonts only, nuclear hypertrophy.

DIAGNOSIS: A *Plasmodium* parasite of Madagascan chamaeleons which produces 10-16 merozoites in schizonts that are smaller than or equal to the size of the host cell nucleus. Merozoites are typically arranged peripherally around 3 or 4 clusters of dark pigment granules which tend to lie centrally in the schizont. Gametocytes are usually oval or elongate, slightly larger than the host cell nucleus, on average. Both schizonts and gametocytes tend to occupy polar positions in erythrocytes. Infected cells are enlarged; those containing schizonts show enlarged nuclei. No nuclear hypertrophy is produced by gametocyte presence.

TYPE LOCALITY: Périnet, Madagascar (48° 26'E, 18° 55'S).

TYPE HOST: *Chamaeleo brevicornis*, Gunther, 1879.

GEOGRAPHIC DISTRIBUTION: Known only from the type locality.



FIGS. 75-89. — *Plasmodium brygooi* n. sp. from *C. brevicornis*.

75-79: schizonts.

80: segmenter with 16 merozoites.

81: young gametocytes.

82, 83: macrogametocytes.

84-89: microgametocytes.

FIG. 90. — Probable *Leishmania* amastigotes in thrombocyte. Photographed from restained type slide. Host cells in figures 75, 77 and 79 are proerythrocytes, remainder erythrocytes. Vertical bars represent 10 μm.

Discussion

Plasmodium brygooi is distinguished from *P. robinsoni* by smaller schizont size and fewer merozoites. *Plasmodium robinsoni* schizonts are 2-4 times the size of host cell nuclei; those of *P. brygooi* are 0.5-1.2 times host cell nucleus size. Merozoites number 40-74 in *P. robinsoni*, but only 10-16 in *P. brygooi*. Merozoites

of *P. brygooi* are arranged peripherally around an approximately central group of pigment clusters. In *P. robinsoni* merozoites fill the entire schizont, with pigment usually coalesced into a single prominent mass. The smaller *P. robinsoni* sexual forms can be confused with those of *P. brygooi*. In both hosts, some *P. robinsoni* gametocytes have LW values of 72-75 μm^2; the smallest *P. brygooi* gametocytes were 66 μm^2. The maximum LW of *P. brygooi* gametocytes, 126 μm^2, is far lower than that of *P. robinsoni* in either host (170-221 μm^2). The greatest difference in gametocytes is that of size (LW) relative to infected erythrocyte nucleus size (LW): over 80 % of *P. brygooi* gametocytes are less than twice the host nucleus size. In contrast, over 90 % of *P. robinsoni* gametocytes in both hosts exceed host cell nucleus size by twice or greater. *Plasmodium brygooi* gametocytes tend to occupy polar or lateropolar positions in their host cells (98 %), in comparison to *P. robinsoni* gametocytes which are often lateral to the nucleus (23 %) and sometimes virtually fill the host erythrocyte.

The brief description of *P. robinsoni* by Brygoo (1962) fits well with the present observations. He found mature schizonts containing 50-70 nuclei in the type host, *C. brevicornis*. The only mature schizont found on the type slide showed 47 nuclei, but in *C. parsoni* 40-74 nuclei were present. Brygoo also found gametocyte shape highly variable, with length attaining and frequently surpassing three-quarters of the host cell length. Brygoo described the presence of one or two vacuoles in trophozoites. These were not seen in the very few trophozoites present on the type slide, nor in the infection from *C. parsoni*, but pigmentation of young trophozoites, as mentioned by Brygoo, was evident in the type infection. Pigment was not detected in the smaller trophozoites of *P. robinsoni* in *C. parsoni*.

During study of the type slide of *P. brygooi* from *Chamaeleo brevicornis*, a single thrombocyte was found which appeared to contain three *Leishmania* amastigotes *(fig. 90)*. These were very similar to amastigotes reported by Telford (1979) from Pakistani geckoes and agamid lizards, and may indicate the presence of a saurian *Leishmania* in Madagascar.

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