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BILLBRAYA AUSTRALIS N. GEN. N. SP. FROM THE SOUTH AUSTRALIAN GECKO *PHYLLODACTYLUS MARMORATUS*

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SUMMARY

Billbraya australis n. gen. n. sp. (Plasmodiidae; Haemosporidia) is described from the blood of the South Australian gecko *Phyllodactylus marmoratus*. Parasitaemia in one of the two infected hosts was followed for 6 months. The blood infection developed in two distinct stages: first, merogony, lasting about a month following capture, with massive infections of up to 12 parasites per erythrocyte in 95 % of the circulating erythrocytes, resulting in severe anaemia; next, a massive gamogony, lasting another month. The mature gametocytes remained in the blood until observation was concluded 4 months later. Exoerythrocytic merogony was observed in a circulatory monocyte. Recrudescence from exoerythrocytic divisions yielded only gametocytes.

RÉSUMÉ : **_Billbraya australis_ n. gen. n. sp. parasite du Gecko sud-australien _Phyllodactylus marmoratus_.**

Billbraya australis n. gen. n. sp. (Plasmodiidae; Haemosporidia) est décrit dans le sang d'un Gecko sud-australien *Phyllodactylus marmoratus*. La parasitémie de l'un des deux hôtes infectés a été suivie pendant 6 mois. L'infection sanguine a évolué en deux étapes distinctes : d'abord la mérogonie pendant environ un mois après la capture, avec des infections massives allant jusqu'à 12 parasites par érythrocyte dans 95 % des érythrocytes du sang, entraînant une forte anémie; puis une forte gamogonie, pendant le mois suivant. Les gamétocytes mûrs sont restés présents dans le sang jusqu'à la fin de l'observation, 4 mois plus tard. Un méronte exo-érythrocytaire a été observé dans un monocyte du sang. Les recrudescences des gamétocytes proviennent des divisions exo-érythrocytaires.

INTRODUCTION

In the present communication we describe a new genus and species of haemosporidian from the south-Australian gecko *Phyllodactylus marmoratus* and discuss its relationship with other reptile haemosporidians.

MATERIAL AND METHODS

Data from two infected geckoes were obtained. One (MB291) was collected in 1981 and examined by necropsy soon after collection. The other (PD-P) was collected in 1988 and kept in captivity for a follow-up of the parasitaemia, for about 6 months. Blood was obtained by clipping the tip of the toe. Smears were air-dried, fixed in methanol and stained for 1 hour in diluted (10 %) Giemsa. Differential counts for erythrocyte types and infection were carried out for at least 1.000 erythrocytes. At the end of the follow-up period, necropsy was performed and smears prepared from visceral tissues were fixed and stained as blood smears. For histology, tissues were fixed in Carnoy's solution, embedded in paraffin and the prepared sections were stained by the Giemsa colophonium method. All recorded measurements are in microns.

RESULTS

Infection was found in two *Phyllodactylus marmoratus* from south Australia: in one (gecko MB291) of the four collected at Blowhole Creek, Fleurien Peninsula, on 21 novembre 1981, and in one (gecko PD-P) out of the 22 collected from Red River gum trees along the Marne river at Cambraii on 2 novembre 1988. All 6 *P. marmoratus* collected from gum trees at Maccles fields were negative (two of them were infected with *Pirhemocyton* virus).

In gecko MB291 infection (34 % parasitaemia) was comprised of mature gametocytes and a few trophozoites. At capture, gecko PD-P exhibited an infection comprised of

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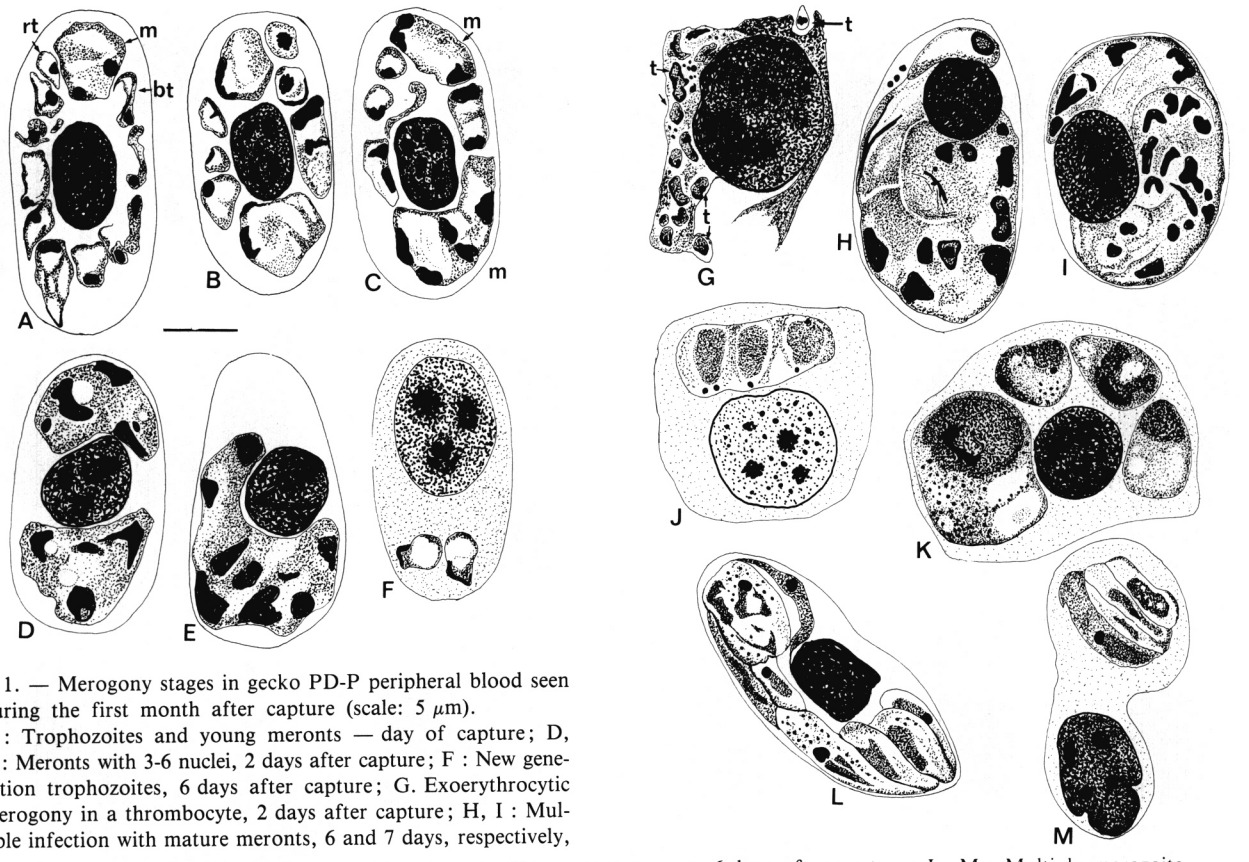

FIG. 1. — Merogony stages in gecko PD-P peripheral blood seen during the first month after capture (scale: 5 μm).
A-C : Trophozoites and young meronts — day of capture; D, E : Meronts with 3-6 nuclei, 2 days after capture; F : New generation trophozoites, 6 days after capture; G. Exoerythrocytic merogony in a thrombocyte, 2 days after capture; H, I : Multiple infection with mature meronts, 6 and 7 days, respectively, after capture; J : Meront in proerythrocyte; K : Young gametocytes, 6 days after capture; L, M : Multiple merozoite (or early gametocyte) infection, 7 days after capture.

massive erythrocytic merogony, with 95 % of the mature erythrocytes infected.

DESCRIPTION

A — Definitions:

Definition of Billbraya *n. gen.:*

Plasmodiidae; morphology comparable to that of *Plasmodium* species; development in the red blood cells of lizard following two distinct consecutive stages: — abundant merogony, causing important anaemia — followed by gametogony. Maturation of gametocytes very long (> 1 month), completed after repair of anaemia. Recrudescences of gametocytaemia stemming from exoerythrocytic divisions. Exoerythrocytic merogony seen in the circulatory monocytes. Gametocytes round, oval or oblong but never of the halteridian type.

Etymology: The new genus is named in honor of Robert (Bill) S. Bray, parasitologist, protozoologist and a good friend.

Type species: Billbraya australis: *n. sp.*

Host: *Phyllodactylus marmoratus.*

Type locality: Cambraii, South Australia.

Type specimen: From a slide deposited in the Museum National d'Histoire Naturelle (N° PXII 84).

B — Morphology

Trophozoites: Ring-form 1,6-5,6 × 1,2-2,4 and band-form 1,6-5,6 × 0,3-0,5 trophozoites with blue cytoplasm and a red nucleus *(fig. 1, A)* were the most abundant. Later stage, trophozoites with blue pink cytoplasm of variable density *(fig. 1, B, C; 4, B, C)* were 4,8 × 1,6-4,8. Only a few trophozoites were found at later stages of infection, often mixed with more advanced stages, including gametocytes. These trophozoites were round 2-2,4 × 1,6-2, or oblong 2,4-4 × 0,8-2,0, and had a narrow cytoplasm with one or two red caryosomes stretched along the margins of a large central vacuole *(fig. 1, F; 5, J, K).*

Meronts: Meronts with one or two nuclei were either small, 3,2-6,4 × 1,6-4,0 and reminiscent of ring stages, or *(fig. 1, B, C)* larger 6,4-9,6 × 2,4-6,4 with blue cytoplasm of variable density *(fig. 4, B, C, D, E).* The smaller meronts occurred predominantly in mature erythrocytes *(fig. 1, A-C),* while the larger ones occurred also commonly

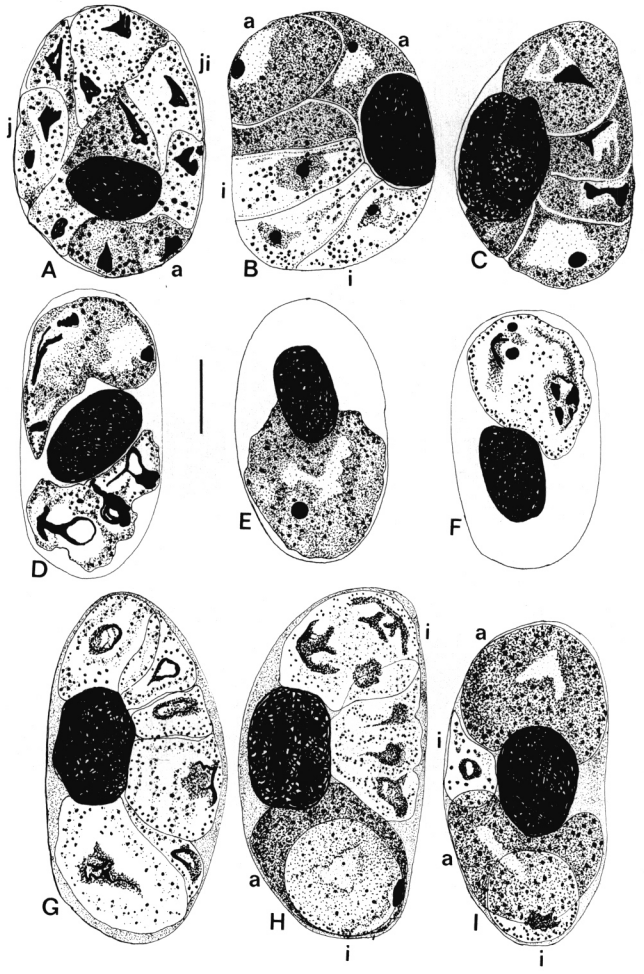

FIG. 2. — Infection seen in gecko PD-P peripheral blood during the second month after capture (scale 5 μm). A-C: Multiple infection with young micro (i) and macrogametocytes (a), 40 days after capture; D : mature meronts, 40 days after capture; E : Macrogametocyte, 40 days after capture; F : Microgametocyte, 40 days after capture. G-I : Young and ongrowing gametocytes, 56 days after capture.

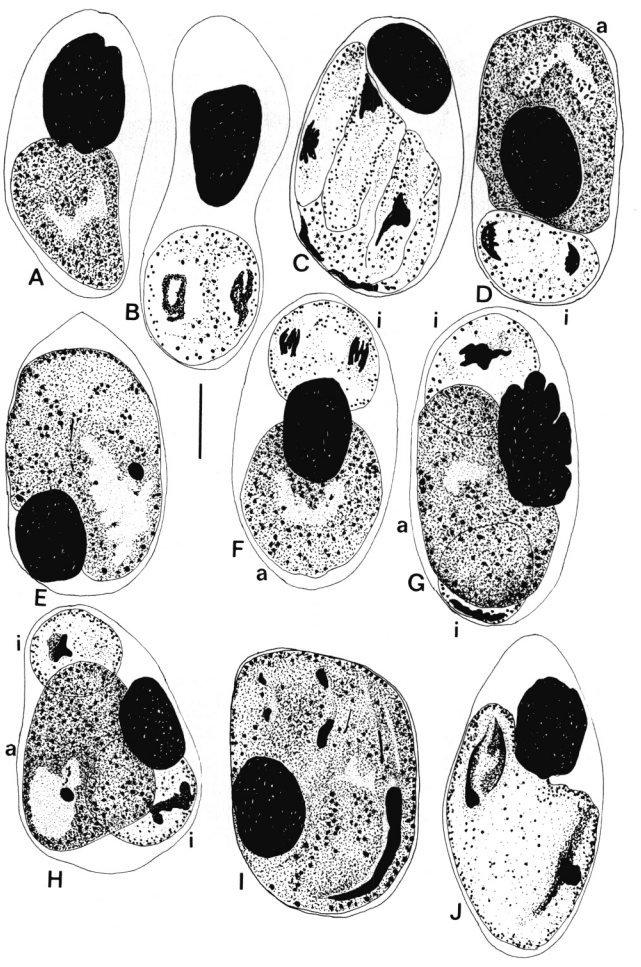

FIG. 3. — Early and late stage gametocytes in geckoes PD-P (A-H) and MB291 (I, J) peripheral blood (scale 5 μm). A, B : Macrogametocyte and microgametocyte, 56 days after capture : C : Multiple infection by young microgametocytess, 115 days after capture; D : Micro (i) and macrogametocyte (a) coinfection, 115 days after capture; E, F : Gametocytes, 127 days after capture; G, H : Gametocytes, 153 days after capture; I, J : Macro and microgametocytes from gecko MB291.

in premature erythrocytes. Meronts with 3-5 nuclei 5,6-7,2 × 2,8-4,8 and with 6-8 nuclei 8,0-11,2 × 5,6-8,8 occurred in premature and mature erythrocytes. These meronts had patchy blue-pink cytoplasm of variable density, a variable quantity of very fine granules of black pigment, and sometimes azurophilic granules as well *(fig. 1,C-E, H-J; fig. 2, D; (fig. 4, B-I)*. Meronts segmenting into merozoites were not found.

Exoerythrocytic meronts: One mature meront 8,0 × 2,4 with 11-12 nuclei was found in a circulating thrombocyte *(fig. 1, G)*.

Young gametocytes: Young gametocytes 4,8-9,6 × 4,0-6,4, often occurred together with meronts and trophozoites in the same immature or mature erythrocytes. By the 2nd week of capture *(fig. 1, L, M)* a few mature erythrocytes were seen loaded with 5-12 young gametocytes. By the 5th week of follow-up, many more were observed. The young gametocytes were either oblong, 4,8-9,6 × 1,6-2,4, or stout, 7,2-8,0 × 4,8-8,0 *(fig. 1; (fig. 2, A-C; 4, K; 5, A-C)*. They could already be differentiated by their pink or blue cytoplasm into microgametocytes and macrogametocytes *(fig. 4, K; 5, A-C)*. Gametocytes of both sexes occupied the same erythrocyte *(fig. 2, A, B, H, I)*. Both sexes contained a large red caryosome and a fair amount of fine granules of black pigment. In the larger ones, particularly the macrogametocytes, the pink nuclear zone was already distinct and contained a central or marginal caryosome *(fig. 2, B, C)*.

Mature gametocytes: Microgametocytes were either

TABLE I. — *Size of gametocytes in subsequent blood samples.*

MONTH	MACROGAMETOCYTES		MICROGAMETOCYTES	
	mean	range	mean	range
December	9,6x8,8	8,8-11,2x4,8-10,4	7,5x5,8	5,6-9,6x2,4-7,2
Feb.-Mar.	13,7x7,8	10,4-19,2x5,6-11,2	8,5x7,0	5,6-12,8x4,0-9,6
April	14,9x7,8	12,0-18,4x7,2-11,2	9,2x7,0	5,6-15,2x4,0-8,8
	(n=14; 29;16)		(n=8; 17; 13)	

TABLE II. — *Course of infection in the gecko's blood: overall levels of parasitaemia vs. infection levels in mature and immature erythrocytes (RBC), all counts were made per 1.000 erythrocytes.*

DATES	PARASITES /1000 RBC	INFECTED RBC /1000	MATURE RBC /1000	PARAS. /1000 MATURE RBC	INF. MATURE RBC/1000	PARAS./1000 IMMAT. RBC	INF. IMMAT. RBC/1000
2.11	990	180	190	5915	950	40	30
4.11	720	160	120	5180	890	80	50
8.11	450	120	150	2630	610	30	20
9.11	310	90	160	1760	350	40	40
5.12	740	350	770	940	430	80	70
12.12	590	280	930	640	310	0	0
28.12	680	320	940	720	330	0	0
29.1	60	30	999				
9.2	54	31	999				
20.2	80	44	999				
22.2	59	40	999				
25.2	33	23	999				
8.3	18	14	999				
22.3	21	16	999				
4.4	23	15	999				
17.4	15	10	999				
24.4	14	13	999				

round, oval or oblong, 5,9-15,2 × 2,4-9,6; their pale-pink cytoplasm contained two chromatin bodies — the caryosome and the accessory granule — and fine pigment granules *(fig. 2, F; 3, B, D, F, J; 4, K; 5, A, B, D, G)*. Macrogametocytes were usually oblong, less frequently oval, 8,8-19,2 × 4,8-11,2, and contained blue cytoplasm, a diffused red nuclear zone and scattered fine black pigment. The caryosome was only seen in some of the macrogametocytes *(fig. 2, E; 3, A, D, E-I; 5, D-F, H-K)*.

Micro and macrogametocytes proceeded growing and only reached their maximal sizes four months after the initial differentiation was observed *(table I)*.

Effect on the host cell: Even when massively infected with trophozoites and meronts, mature erythrocytes only exceptionally increased in size (mean = 18,2 × 9,4; n = 13 *(fig. 1, A-E; 4, A, E, F)* compared with a mean of 17,9 × 10,8; n = 14 of uninfected erythrocytes) or became deformed *(fig. 4, C, D)*. Erythrocytes also retained their

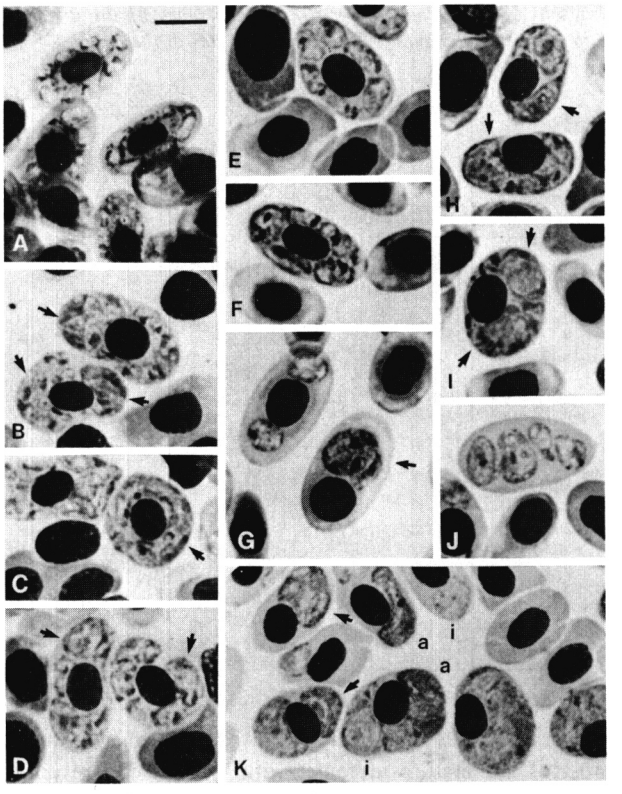

FIG. 4. — Peripheral blood from gecko PD-P (scale 10 μm). A : Trophozoite infection, 2 days after capture; B-D : Young and premature (arrows) meronts, 6 days after capture; E-J : Young (E, F) and mature meronts (arrows, G-I), and gametocytes (J), 7 days after capture; K : Micro (a) and macrogametocytes (a) and mature meronts (arrows), 33 days after capture.

size when infected with one or a couple of mature gametocytes (mean = 17,4 × 10,0; n = 23), a change in size being only very exceptional *(fig. 3, A, B; 5, A, B)*. However erythrocytes became distinctly enlarged when infected by multiple young gametocytes (mean = 19,1 × 11,4; n = 10 when infected mainly with macrogametocytes, and 20,2 × 10,7; n = 17 when infected mainly with microgametocytes) *(fig. 2; 4, K; 5, C)*. The nucleus central and was only rarely displaced in erythrocytes infected with trophozoites, meronts or young gametocytes. When infected with a single or several mature gametocytes, the nucleus was occasionally displaced laterally or, when gametocytes were oval-oblong, to polar position *(fig. 3; 5, E-I)*.

C — Course of infection

Merogony in the first month of infection: The blood of gecko PD-P, examined at capture and during the first week thereafter, consisted predominantly (81-88 %) of immature erythrocytes: proerythrocytes and erythroblasts. Most of the mature erythrocytes were infected with up to 12 young parasites. Infection was initially comprised of early band- and ring-form trophozoites and young meronts *(table II; fig. 4, A-F)*. Level of parasitaemia in the imma-

TABLE III. — *Frequencies (in %) of occurrence of the different developmental stages in blood erythrocytes in the course of the gecko's infection.*

DATES	TROPHOZ.	MERONT-2	MERONT-4	MERONT-5+	NON-DIFF. GAMETOC.	JUVENILE MICROG.	JUVENILE MACROG.	MATURE MICROG.	MATURE MACROG.
2.11	55	36	6	2					
4.11	76	16	2	4					
8.11	26	24	18	16	13				
9.11	4	50	14	9	21				
5.12	0,5	0	0,5	2	12	7	77		
12.12	0,1	0,3	0	1,2	1,4	39	57		
28.12	0,3	0	0	0	0	17	2	43	36
29.1						8	0,7	57	34
9.2						7	0	57	36
20.2	27					35	1,8	1,8	34
22.2						6	1,3	46	45
25.2						3	0	54	41
8.3						8	0	54	37
22.3	1					16	0	51	32
4.4						6	0,8	70	22
17.4						1,2	0	65	34
24.4								62	38

ture erythrocytes ranged from only 2-5 %, but contained most of the mature meronts (with 4-8 nuclei) prevailing at this stage of infection in the gecko's blood. The number of parasites in the immature erythrocytes only exceptionally exceeded two. Initially the levels of parasitaemia of mature erythrocytes was 95 % with 5.915 parasites per 1.000 mature erythrocytes, but by the end of the first week of captivity it had already dropped to 35 %, with 1.790 parasites per 1.000 mature erythrocytes. The levels of infection of immature erythrocytes did not show a parallel decline *(table II)*. During this week there was a gradual shift in abundance from trophozoites to dividing meronts: prevalence of trophozoites declined to 4 %, while the relative prevalence of two-nucleate meronts and meronts with 4-8 nuclei rose to 50 and 23 % respectively, of total parasites *(table III, fig. 4, G-I)*.

Early period of gametocytogony: Young gametocytes, which still could not be differentiated into micro and macrogametocytes had already appeared at the end of the first week of captivity, reaching a relative prevalence of 22 %. In some erythrocytes they appeared in closely apposed batches, as if they had been generated from a common meront within the erythrocyte *(fig. 1, K; 4, J)*.

When follow-up was resumed the following month (33 days after capture) mature erythrocytes had regained dominance, comprising 77 %, and ultimately, 62 days after capture, 94 % of the erythrocyte-count. They were loaded with 6-11 undifferentiated gametocytes or young micro and macrogametocytes *(tables II, III; fig. 4, K; 5, A-C)*. The level of parasitaemia in mature erythrocytes was 43-31 % (940-640 parasites per 1.000 erythrocytes). Trophozoites and

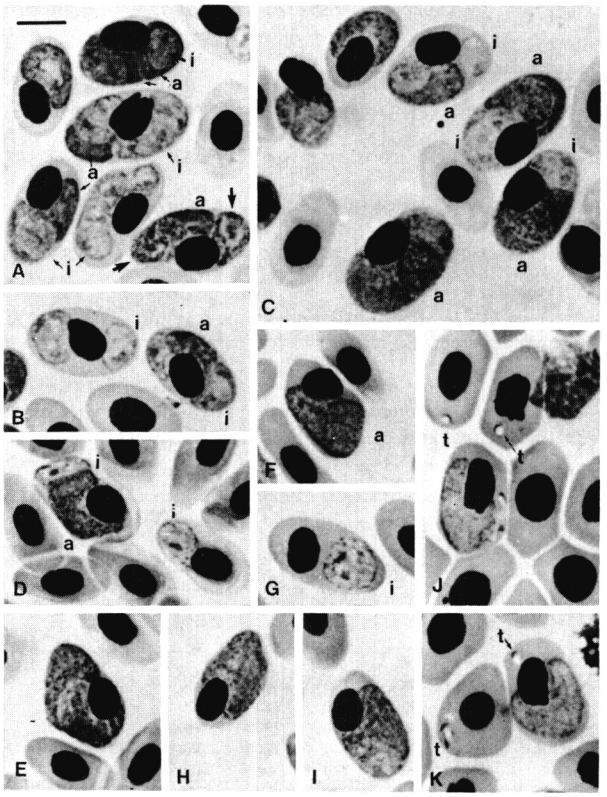

FIG. 5. — Peripheral blood of geckoes PD-P (A-I) and MB291 (J, K) (scale 10 μm).
A, B : Micro (i) and macrogametocytes (a) and late meront (arrow), 40 days after capture; C : Gametocytes 56 days after capture; D, E : Gametocytes, 88 days after capture; F, G : Micro and macrogametocytes, 115 days after capture; H, I : Macrogametocytes 166 days after capture; J, K : Macrogametocytes and new trophozoites (t) in Gecko MB291 blood.

meronts (mainly with four or more nuclei) were very rare (0,4 and 3 % respectively) *(fig. 5, A)*. Large, apparently mature gametocytes first appeared on the 52nd day after capture.

Later period of the gametocyte infection (the chronic stage): The level of parasitaemia by the end of the third month of captivity declined to 3 % (60 parasites per 1.000 erythrocytes, *(table II)*. By then the infection consisted entirely of large, mature gametocytes *(fig. 5, D-K)*. Infected erythrocytes often contained one, and exceptionally more than two, gametocytes *(fig. 5, D, E)*, usually a single macrogametocyte accompanied by one or two smaller size microgametocytes. Occasional recrudescence of trophozoites, and reappearance of young gametocytes occurred but never merogony stages *(table III)*. At the end of the 6th month observation period, when the gecko was sacrificed for necropsy, levels of parasitaemia declined to 0,9-1,3 % (10-14 parasites per 1.000 erythrocytes, *table II)*.

Macrogametocytes outnumbered microgametocytes (10-1,

4 to 1), in the early period of gametocytaemia (33-40 days after capture). After a brief period of approximate parity, the ratio was reversed to a predominance of microgametocytes (1,6-3,3 to 1). Follow-up data suggest that the occurrence of macrogametocytes preceeded that of microgametocytes, while subsequent juvenile microgametocytes substantially outnumered young macrogametocytes *(table III).*

DISCUSSION

To date, two species of *Plasmodium* in Australia have been described by Mackerras (1961): *P. egerinae* from the skink *Egerina major* and *P. giganteum australis* from *Amphibolurus barbatus.* They differ from *B. australis* by having larger merozoite progenies and by the structural details of their gametocytes. The gametocytes of *Haemoproteus gehyrae,* found in the north Australian gecko *Gehyra dubia* (Paperna and Landau, in press), resemble the gametocytes of the presently described species but differ by their bigger size, and some minor morphological differences like the caryosome body, constantly present in the nucleus of macrogametocytes of *H. gehyrae,* rarely seen in *B. australis.*

Follow-up of parasitaemia in *G. dubia* for 10 months revealed only gametocytes. This parasite was therefore classified in the genus *Haemoproteus,* but if it later appears that we missed the merogonic stages, it might well be the same parasite as *B. australis.* Late stages of a saurian *Plasmodium,* consisting entirely of gametocytes, have been confused in the past with *Haemoproteus* (Wenyon, 1915, Irtube and Gonzales, 1921; Bray, 1959). Taking into account the present data, the parasite must nonetheless be accepted as a species of *Haemoproteus.*

The taxonomy of Haemoproteidae has been established by taking into account the morphological features of the blood stages, and biological characteristics such as site and dimensions of tissue meronts, vectors and vertebrate hosts. Most genera are commonly accepted, and species, when their life cycle is known, can easily be classified into one of the existing genera. In the family Plasmodiidae, however, all pigmented parasites of vertebrate hosts which undergo merogony in the circulating blood erythrocytes have been grouped into a single genus, *Plasmodium,* without taking into account the characteristics of their exoerythrocytic cycle, the course of their merogony and gamogony in the blood, their vectors and their vertebrate hosts. Consequently, reptilian plasmodia which have their exoerythrocytic cycle in the reticulo-endothelial tissue and are transmitted by Psychodidae and Ceratopogonidae, and mammalian plasmodia which have their exoerythrocytic cycle in the hepatocytes and are transmitted by anopheline mosquitoes are grouped in the same genus. Attempts to classify species into several genera, for example into *Plasmodium falciparum, Laverania* Grassi and Feletti, 1890, or *Haemamoeba* and plasmodia of reptiles into *Haemamoeba* (Bray, 1959), have not been followed.

The presently described plasmodiid parasites, in our opinion, cannot be accomodated in the genus *Plasmodium,* and we propose their placement in a new genus, *Billbraya.* The parasites undergo merogonic division in the RBCs like *Plasmodium,* but resemble haemoproteids in having their gamogony separate, and consequent to merogony. Erythrocytic merogony is restricted to the early stage of the infection, while later recrudescence, like the haemoproteids, consists only of gametocytes, apparently generated from exoerythrocytic merogony. In all species of *Plasmodium* from reptiles (Ayala, 1978, Telford, 1984) continuous or relapsing erythrocytic merogony occurs concurrently with gametocytes. Another feature characteristic to the presently described parasite is the extreme level of infection in the erythrocytic merogony stage. The hyperinfection involves most circulating erythrocytes. An anaemic condition evidenced by the predominance of proerythrocytes, which develops during the merogonic parasitaemia phase, appears to result directly from the massive loss of infected erythrocytes. The anaemia regressed by the end of erythrocytic merogony. Anaemia with an extreme increase in the rate of immature erythrocytes, to levels comparable to those presently described (83 %), has been reported in *P. colombiensis,* but the level of infection during merogony did not exceed 613 parasites per 1.000 erythrocytes (Ayala and Spain, 1976). Infection levels during merogony in *P. sasai* were higher, but during the resulting anaemia ratios of immature erythrocytes remained in the range of 24-31 % of the total count (Telford, 1972).

The hematological condition occurring durint the merogony stage in gecko resembles a state of crisis found in mammalian malaria. The condition differs however on three important points: *a*) It is a natural phenomenon, unlike in mammals where it is laboratory induced in experimental hosts. *b*) It is prolonged (over a month) rather than brief, as in mammals. *c*) Its end marks the onset of transmissibility, while in mammals, a state of crisis suppresses gametocyte fertility (Landau *et al.,* 1989, Bastien *et al.,* 1987).

The two types of development in reptilian haemosporidia, either asynchronous, *i. e.* with several stages present simultaneously or synchronous, with either meronts or gametocytes in the blood, represent two alternative transmission strategies. In the first case, transmission can theoretically be continuous. In the second, it is likely to be restricted, often seasonal, the blood being flooded with gametocytes during the transmission period. We feel that these biological differences are important because they reflect a diversity in transmission and should therefore be taken into account by the organism's taxonomy.

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