

## **FALLISIA COPEMANI N. SP. (HAEMOSPORIDIA: GARNIIDAE) FROM THE AUSTRALIAN SKINK *CARLIA RHOMBOIDALIS***

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### SUMMARY

*Fallisia copemani* n. sp. is described from the circulatory blood lymphocytes and thrombocytes of the skink *Carlia rhomboidalis* from the rain forest of north Queensland, Australia. Parasites occur predominantly in lymphocytes and enlarge the infected cells. Meronts divide into 12-32 merozoites. In heavy infection up to

85 % of the lymphocytes were estimated to have become infected. Macrogametocytes always outnumbered microgametocytes. Double infections with identical and different stages were common at times in 30-60 % of the infected cells.

### RÉSUMÉ : *Fallisia copemani* n. sp. (Hémosporidie, Garniidae) chez le Scinque australien *Carlia rhomboidalis*.

Description de *Fallisia copemani* n. sp. dans les lymphocytes circulants et les thrombocytes du scinque *Carlia rhomboidalis* capturé dans la forêt humide du Nord Queensland, Australie. Les parasites prédominent dans les lymphocytes qui deviennent hypertrophiés. Les schizontes produisent 12-32 mérozoïtes. Lorsque

la parasitémie est élevée, le taux d'infection des lymphocytes est estimé à 85 %. Les macrogamétocytes sont toujours plus nombreux que les microgamétocytes. Des infections doubles par le même stade ou par deux stades différents ont été parfois observées dans 30-60 % des cellules parasitées.

### INTRODUCTION

Examination of blood from the skink *Carlia rhomboidalis* (Peters) from the rain forest of north Queensland, Australia, revealed infection by a hemosporidian of the genus *Fallisia* Lainson, Landau and Shaw in the leucocytes and thrombocytes. Species of *Fallisia* were described from iguanas and skinks from South America (Lainson, Landau and Shaw, 1974; Lainson, Shaw and Landau, 1975) and from an agamid lizard in Thailand (Telford, 1986). Unidentified hemosporidian in circulating lymphocytes and thrombocytes which could have been a species of *Fallisia* have been reported by Thompson and Hart (1946) from a skink — *Leiopisma fuscum* from New Guinea islands area (= *Carlia fusca* (Duméril and Bibron), S. Donnellan, South Australia Museum, Adelaide and H. Marx, Field Museum of Natural History, Chicago, Illinois, USA, personal communications).

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### MATERIALS AND METHODS

Captured skinks *C. rhomboidalis* were taken to the laboratory and maintained on a varied insect (mostly termite) diet. Only one infected skink was available for this study. Blood was obtained by clipping the tip of the tail, smears or touch preparations were air dried, fixed in absolute methanol and stained in Giemsa (diluted 1:7 in a phosphate buffer of pH 6,8) for 15 minutes. Levels of parasitaemia were determined by counts of infected cells per number of erythrocytes (RBC's) (per 10 000) and per uninfected lymphocytes (as % infected). To follow the development of the parasite and the parasitaemia, the infected skink was bled every 3-15 days (tables I, II). Because of the small size of the host more frequent blood examinations were not attempted to prevent extreme stress. The infected skink was killed 71 days after capture, and smears from the lung, liver and spleen were taken, fixed in absolute methanol and stained in Giemsa as above. All measurements are in microns.

### RESULTS

Of 6 individuals of *C. rhomboidalis* captured in August 1988 in the low altitudes of the Daintree forest area in north Queensland, only one specimen was found to be infected by both *Fallisia* and a haemogregarine (in the RBC's). The rest, as well as 3 additional specimens of the same species captured in the same locality in July, 1986 were negative for blood parasites.

TABLE I. — Follow-up of parasitaemia in an infected skink (per 10 000 RBCs).

Date	Infected cells:		Parasites:			Lymphocytes:		Mon. tot.
	Haem.	Fall.	Mer.	Mac.	Mic.	Non inf.	Infec. (%)	
1/9	90	71	< 4	62	24	79	71 (47)	267
4/9	176	142	< 8	134	25	26	142 (84)	195
9/9	160	134	< 13	147	13	75	134 (64)	113
13/9	178	33	< 5	33	5	104	33 (24)	79
26/9	270	22	2	29	3	251	22 (8)	280
5/10	552	56	< 8	56	< 8	120	56 (32)	263
26/10	188	14	< 2	14	< 2	291	14 (5)	94
10/11	1 173	10	4	6	< 1	no data		

Haem.: « *Haemogregarina* » sp.; Fall.: *Fallisia*, all stages; Mer.: Merogony stages; Mac.: Macrogametocytes; Mic.: Microgametocytes; Infec. (%): number and % infected; Non inf.: not infected; Mon. tot.: Total monocyte count.

TABLE II. — Relative frequency in % of developmental stages of *F. copemani* in infected lymphocytes in %.

Date	Mer < 16	Mer > 16	Mac. S.	Mac. M.	Mic. S.	Mic. M.	Mac. and Mic.
1/9	2,2	15,9	52,2	15,9	2,2	0	11,4
4/9	9,0	6,0	48,4	15,1	6,1	3,0	12,1
9/9	14,9	14,9	43,2	8,1	8,1	0	10,8
13/9	3,2	0	74,2	16,1	3,2	0	3,2
26/9	0	4,5	40,9	45,4	0	0	9,0
5/10	0	0	96,1	3,9	0	0	0
26/10	4,7	0	85,7	9,5	0	0	0
10/11	3,0	28,0	69,0	0	0	0	0

Mer. < 16 and > 16: meronts yielding < 16 > merozoites; Mac.: macrogametocytes; Mic.: microgametocytes; M.: multiple infection; S.: single infection; ( $n = 21 - 45$ ).

#### DESCRIPTION OF *Fallisia copemani* n. sp.

##### Host-parasite relationship

Meronts and gametocytes parasitize lymphocytes of the circulatory blood (figs. 1-7) (very rarely the thrombocytes) (app. 0,7 %) (figs. 8; 18 G, H). Stages in cells other than that of the circulatory blood could not be found. Parasites enlarge the lymphocyte, from 4,8-6,4 × 4,8-5,6 to 11,2-16,8 × 8,0-9,6, in meront infections (figs. 1-6) and 11,2-18,4 × 7,2-10,4 in gametocyte infections (figs. 9-17); the extreme values occur in multiple infections. The host cell nucleus often retains its size; in multiple infections, however, it may become compressed, but never becomes indented (figs. 6, 13, 15). The few thrombocytes found infected (figs. 8, 18 G, H) were larger (11,2 × 6,4 in a single infection, 12 × 12 in a double infection) than the uninfected (9,6-10,0 × 4,8-5,2). Infection in the thrombocytes does not cause thickening of the cell membrane.

In the lymphocytes, meronts as well as gametocytes have a smooth rounded outline. They fill the entire cytoplasm and closely appose the host cell nucleus as well as adjacent parasites in multiple infections (figs. 13, 14, 18 I-N). In

the latter, the parasites are located around, or at one side of the host cell nucleus. Adjacent parasites in multiple infections become compressed (figs. 13, 18 N). In the infected thrombocyte, parasites, even in double infections, occupy only part of the host cell cytoplasm and therefore retain their rounded shape (figs. 18 G, H).

##### Morphology

Meronts divide into 12-32 merozoites. The size of the meronts varies with the number of merozoites they produce: meronts of < 17 merozoites, 8,6 × 6,4 (7,2-11,2 × 4,0-8,0;  $n = 10$ ); 17-24 merozoites, 8,9 × 7,8 (8,0-9,6 × 6,4-8,0;  $n = 4$ ); and > 24 merozoites, 9,0 × 7,4 (8,0-10,4 × 6,4-8,0;  $n = 7$ ) (figs. 1-7, 18 A-F). Meront cytoplasm changes in colour during the process of differentiation from blue to pink. Mature merozoites before their release from the host cell (figs. 7, 18 D-F) measured 1,6 × 0,64.

Microgametocytes were 9,1 × 5,25 (6,4-9,6 × 3,6-6,4;  $n = 16$ ) in single infections, and slightly smaller, 8,4 × 4,2 (6,4-9,6 × 3,6-5,6;  $n = 4$ ) in multiple infections (figs. 3, 4, 13, 17, 18 M, N). Microgametocyte cytoplasm stains

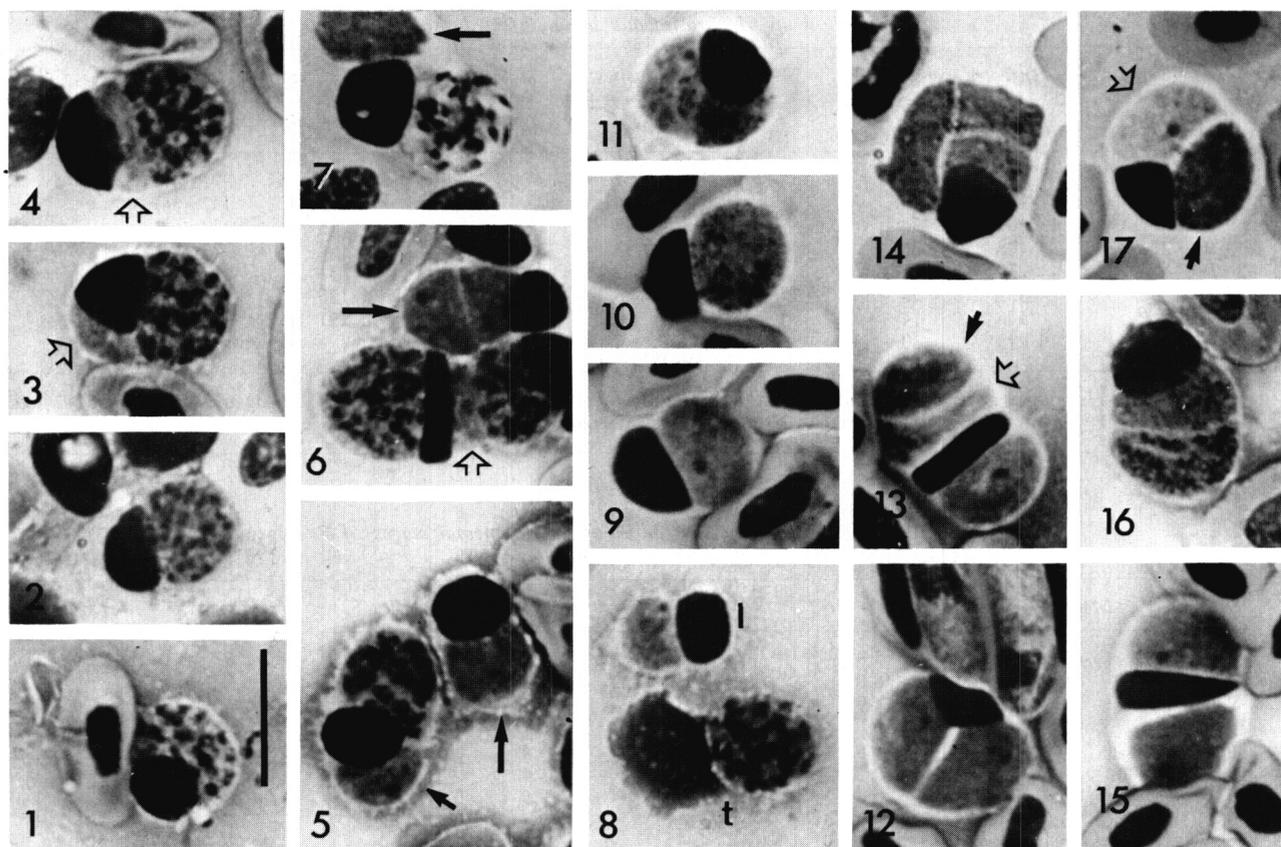


FIG. 1-17. — All infected cells are lymphocytes, except in fig. 8 where one of the host cells (t) is a thrombocyte.

1, 2, 7: meronts; 3, 4: double infection by meronts and microgametocytes (large arrow); 5: double infection by a meront and a macrogametocyte (short arrow) and single infection by a macrogametocyte (long arrow); 6: two lymphocytes infected by meronts and one (long arrow) by two macrogametocytes; 8: a thrombocyte (t) with a meront and a lymphocyte (l) with a macrogametocyte; 9, 10: macrogametocytes; 11: double infection by a meront and a macrogametocyte; 12: double infection by macrogametocytes and also two erythrocytes infected by hemogregarines; 13: triple infection by two macrogametocytes (small arrow) and a microgametocyte (large arrow); 14: triple infections by macrogametocytes; 15, 16: double infections by macrogametocytes; 17: mixed infection by a microgametocyte (large arrow) and a macrogametocyte (small arrow).

pink, and nuclear zone cannot be distinguished except for a large red caryosome. The cytoplasm also contain several red granules.

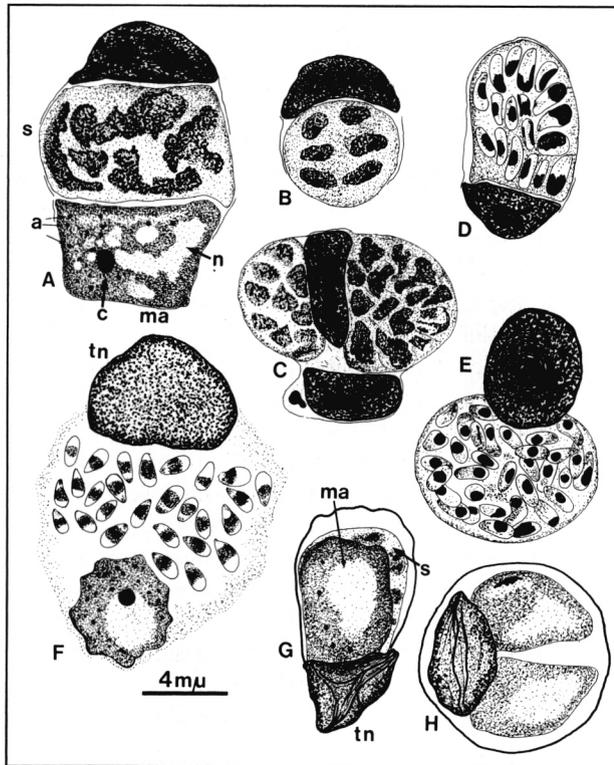
Macrogametocytes were  $7,7 \times 5,4$  ( $6,0-11,2 \times 3,2-7,2$ ;  $n = 20$ ) in single infections (figs. 5, 8-10, 18 I) and  $7,9 \times 4,7$  ( $4,8-11,2 \times 2,8-5,6$ ;  $n = 23$ ) in multiple infections (figs. 5, 6, 11-17, 18 J-N). The blue cytoplasm of the macrogametocyte contains a large central red nuclear zone and varying numbers of red or azurophil granules. A dense red, large, at times fragmented, caryosome, was located within or outside the red nuclear zone.

#### Follow-up of infection

Blood smears were taken within a follow-up period of 71 days (table I). The skink was also concurrently infected with haemogregarines, which were considerably more prevalent in the blood smears than *Fallisia* (90-1 179 parasites per 10 000 RBC's compared with 10-142). High levels of parasitaemia with *Fallisia* were observed only during the first 10 days after capture and later declined. This decrease in the levels of parasitaemia of *Fallisia* was coincident with an increase of RBC's infection by the haemogregarine (table I). In a heavy infection, up to 85 % of the circulating lymphocytes in the peripheral blood were estimated to have become infected. Blood cell counts during high infection (in 47-85 % of lymphocytes, 71-134 infected cells per 10 000 RBC's, table I) suggest depletion in number of circulating lymphocytes (from 120 and higher to below 80/10 000 RBC's). In another non-infected skink of the same species 125 and 222 lymphocytes per 10 000 RBC's were counted. Corresponding counts of monocytes and other leucocytes (granulocytes) in infected and non-infected skinks fluctuated with no particular relationship to lymphocyte counts (table I).

Sequence of development and sex ratio

Trophozoites were never found; meront abundance was always less than 30 % of the parasites present. Gametocytes



occur concurrently with meronts. Macrogametocytes always outnumbered microgametocytes, and the discrepancy between the two widened with the decline in macrogametocyte occurrence (from about 3:1 to 25: < 1). With the decline in the overall level of *Fallisia* infection, meronts and microgametocytes disappear and the infection consists entirely of macrogametocytes. Towards the end of our follow-up monitoring meronts reappear, but not microgametocytes (tables I, II).

#### Double infections

Double infection in lymphocytes, with same or different developmental stages, is common (at times in 30-60 % of

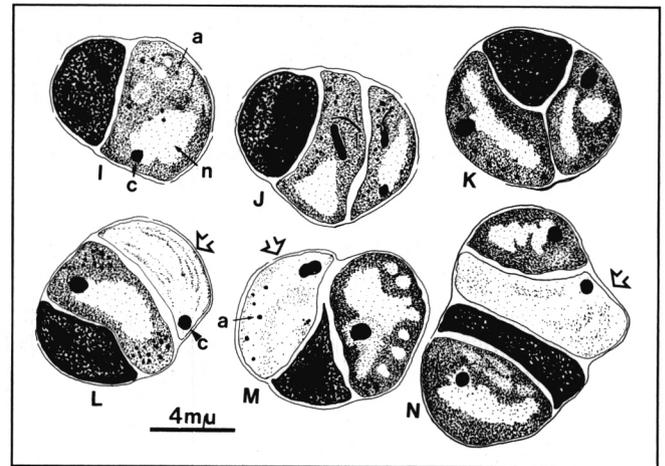


FIG. 18. — All parasites are inside lymphocytes except G and H where they are in thrombocytes. A: immature meront(s) and macrogametocyte (ma); B, C: immature meronts; D, E: mature meronts; F: mature ruptured meront and macrogametocyte; G: multiple infection by macrogametocyte and young meront in a thrombocyte; H: 2 macrogametocytes in a thrombocyte; I, J, K: macrogametocytes; L, M, N: mixed infections by microgametocytes (arrow) and macrogametocytes. (Ma = macrogametocyte; s = schizont; n = nucleus; c = caryosome; a = azurophil granules).

the infected cells). Triple infections occur rarely (< 10 %). Observed frequencies of double and multiple infections of macrogametocytes (table II) usually approximated or were less than the expected probabilities for random pairing (table III). The exceptions are instances of low parasitaemia occurring on the dates 26/9 and 26/10 (table I), where double infection frequencies were considerably higher than expected for random pairing (table III). Mixed macro-microgametocyte infections occurred at much higher rate than the expected probability for random pairing (table III). At times, microgametocytes occurred only in multiple infections with either macrogametocytes or meronts (table II).

#### DISCUSSION

##### Taxonomy

Two genera of haemosporines have been described which infect saurian leucocytes and thrombocytes: *Fallisia* (Lainson, Landau and Shaw, 1974) and *Saurocytozoon* (Lainson and Shaw, 1969). The first differs from the latter in having a merogonous cycle in circulating blood cells (Lainson *et al.*, 1974).

The presently described species differs from *F. effusa* Lainson *et al.*, 1974, *F. simplex* Lainson *et al.*, 1975, *F. audaciosa* Lainson *et al.*, 1975, and *F. siamense* Telford 1986 in being chiefly parasitic in lymphocytes, while all other species infect predominantly or exclusively either thrombocytes or neutrophils.

TABLE III. — Expected probabilities and observed values (in %) of double infections by micro- and macrogametocytes. Relative frequencies of lymphocytes infected by single micro- and macrogametocytes are extrapolated from data from tables I and II.

Date	Macro + Macro		Macro + Microgametocytes	
	P. obser.	P. expc.	P. obser.	P. expc.
1/9	7,52	6,09	5,39	0,20
4/9	12,75	16,71	10,22	1,60
9/9	5,19	7,66	6,92	1,39
13/9	3,86	3,17	0,77	0,01
26/9	3,63	0,10	0,00	0,00
5/10	1,24	2,85	0,00	0,00
26/10	0,43	0,15	0,00	0,00

Other differential characteristics are:

— *F. effusa*: the limiting membrane of thrombocytes parasitized by *F. effusa* becomes much thickened, thus giving the infected cell a cyst-like appearance (Lainson *et al.*, 1974) while that of *F. copemani* always remains thin.

— *F. modesta*: *F. copemani* is larger and, unlike *F. modesta*, does not cause indentation and other distortions to the nucleus of the infected cell. In *F. copemani* the nucleus of the infected cell retains its rounded shape or may become compressed in multiple infection.

Another intralymphocytic-thrombocytic haemosporidian to be considered is an unidentified species, described from *Leiolepispa fuscum* (= *Carlia fusca*) from an island near New Guinea (Thompson and Hart, 1946). Telford (1986) already suggested that this un-named parasite could have been a species of *Fallisia*. There is a close similarity in gametocyte dimensions and structures and in the size of merozoite progeny. Illustrations were however not provided and from the available data the relationship of this haemoprotozoan and *F. copemani* cannot be determined.

#### *Biological affinities and host-parasite relationship*

The course of parasitaemia in *F. copemani* follows the pattern reported for *Plasmodium* in reptilian hosts (Goodwing and Stapleton, 1952; Telford, 1972; Petit, Landau, Boulard, Gomes and Touratier, 1983). After an acute period of high infection, which may last one to three months, it gradually declines over a prolonged period of three months to over one year.

Infections of *F. copemani* have low rates of merogony, even at the peak parasitaemia. Throughout the period of observation, percentages of meronts never exceeded 30 % and were usually considerably lower. Among *Plasmodium* spp. infections, some were shown to be comprised predominantly of gametocytes (in *P. agamae* and *P. lygosomae*, Bray, 1959; Garnahm, 1966), while other consisted predominantly of merogonous stages. Among the latter, in *P. tropiduri*, during the acute period, meronts comprised 60-90 % of all parasite and in some malarial infection, even during the chronic stage, meronts still comprised 20 % and more of the parasites in the circulatory blood (Telford, 1979).

Reappearance of merogony stages toward the end of observation, at the lowest phase of the parasitaemia, may suggest the onset of a relapse which could not be followed since the host was killed.

In *F. copemani*, multiple infections of the lymphocytes consists predominantly of two and only exceptionally more than three parasites. Multiple infections, with up to five parasites, are frequent in *F. effusa* and *F. audaciosa* infections and less so in *F. modesta* (Lainson *et al.*, 1974, 1975). Multiple infections are present in *F. simplex* (Lainson *et al.*, 1975) and according to Telford (1986), absent in *F. siamense* infections.

In *F. modesta* multiple infected cells were termed 'smear

cells' by Lainson *et al.* (1974) as they were exceedingly fragile and tended to rupture in making the blood films and were suggested to be monocytes rather than lymphocytes. In *F. copemani* multiple infected lymphocytes (as well as thrombocytes) retain firm configuration and do not rupture.

The only quantitative data on frequencies of multiple infections and macro-microgametocytes ratio available for comparison are given by Lainson *et al.* (1974) for *F. effusa*: multiple infection abundance in this species increased with overall infection level.

In *F. copemani* double infection frequencies by macrogametocytes were compatible with a probability for random pairing, while mixed sex infection suggests a predilection for invasion of already infected host cells by the opposite-sex merozoite. In some instances microgametocytes could only be found in cells already containing macrogametocytes; it was suggested that merozoites of microgametocytes invade only cells already infected by macrogametocytes.

In *F. copemani*, like in *F. effusa*, macrogametocytes outnumber microgametocytes and likewise microgametocytes disappear from low infections.

In our present studies of saurian *Haemoproteus* (unpublished data) we also found, as in *Fallisia*, disparity in sex ratios, but with a dominance of microgametocytes; as in *Fallisia*, it becomes extreme in the later stage of the chronic phase. This disparity could be the consequence of different timing for the production of microgametocytes and macrogametocytes, or from differences in their survival rates.

In our opinion, this must have great implication upon the course of transmission in nature. In *F. copemani* and in other similar haemosporidia, transmission appears to be restricted to the acute and subacute periods of the infection and cannot be maintained during the chronic phase when disparity between male and female gametocytes becomes extreme.

#### ETYMOLOGY

The new species is named to honour our friend and colleague Dr Bruce Copeman from Queensland, Australia.

*Acknowledgments.* — Work in Australia was carried out in Dr B. Copeman's laboratory in the Graduate School for Tropical Veterinary Sciences at James Cook University, Queensland, and the authors wish to thank Dr Copeman for his hospitality, encouragement and support. We would like also to thank Dr Steve Donnellan from the South Australian Museum, Adelaide, for identifying the skinks. We wish to thank Dr Nathalie Yonow for the editing the manuscript.

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