# Development of *Hepatozoon caimani* (Carini, 1909) Pessôa, De Biasi & De Souza, 1972 in the Caiman *Caiman c. crocodilus*, the Frog *Rana catesbeiana* and the Mosquito *Culex fatigans*

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*The sporogony of* Hepatozoon caimani *has been studied, by light microscopy, in the mosquito* Culex fatigans *fed on specimens of the caiman* Caiman c. crocodilus *showing gametocytes in their peripheral blood. Sporonts iniciate development in the space between the epithelium of the insect gut and the elastic membrane covering the haemocoele surface of the stomach. Sporulating oocysts are clustered on the gut, still invested by the gut surface membrane. Fully mature oocysts were first seen 21 days after the blood-meal. No sporogonic stages were found in some unidentified leeches fed on an infected caiman, up to 30 days following the blood-meal. When mosquitoes containing mature oocysts were fed to frogs* (Leptodactylus fuscus *and* Rana catesbeiana), *cysts containing cystozoites developed in the internal organs, principally the liver. Feeding these frogs to farm-bred caimans resulted in the appearance of gametocytes in their peripheral blood at some time between 59 and 79 days later, and the development of tissue cysts in the liver, spleen, lungs and kidneys. Transmission of the parasite was also obtained by feeding young caimans with infected mosquitoes and it is suggested that both methods occur in nature. The finding of similar cysts containing cystozoites in the semi-aquatic lizard* Neusticurus bicarinatus, *experimentally fed with infected* C. fatigans, *suggests that other secondary hosts may be involved.*

Key words: *Hepatozoon caimani* - life-cycle - *Caiman c. crocodilus* - *Caiman c. yacare* - *Melanosuchus niger* - caimans - *Rana catesbeiana* - *Leptodactylus fuscus* - frogs - *Culex fatigans* - Brazil

For many years, all haemogregarines recorded in the blood of crocodilians were assigned to the genus *Haemogregarina* under the following specific names: *Hg. hankini* Simond, 1901 of the Indian ghavial *Gavialis gangeticus; Hg. crocodilinorum* Börner, 1901 in *Crocodylus acutus* and *Alligator mississipiensis* from North America; *Hg. caimani* Carini, 1909 in *Caiman latirostris* from Brazil; *Hg. pettiti* Thiroux, 1910 in *Cr. niloticus* from Africa; *Hg. serrei* Phisalix, 1914 of *Paleosuchus trigonatus* from South America; *Hg. sheppardi* Santos Dias, 1952 in *Cr. niloticus* from Africa; and unnamed species of *Haemogregarina* in *Cr. porosus* from Sri Lanka and *Cr. palustris* from Sumatra (Wenyon 1926, Levine 1988).

Chatton and Roubaud (1913) described the sporogony of an *Hepatozoon* sp., in wild-caught tsetse-flies, *Glossina palpalis*, in Africa and suspected that the parasite originated from either a lizard or a crocodile on which the insects had fed. It remained for Hoare (1932), however, to show that similar multisporocystic oocysts developed in laboratory-bred *G. palpalis* fed on *Cr. niloticus* that had haemogregarines in their blood. As a result he amended the name of the parasite to *Hepatozoon pettiti* (Thiroux 1910). In recent years the suggestion has been made that all crocodilian haemogregarines should be transferred to the genus *Hepatozoon* (Siddall 1995, Smith 1996), although *H. pettiti* and *H. caimani* appear be be the only parasites for which there is supportive evidence from the demonstration of the sporogonic stages, characteristic of that genus (Hoare, 1932, Pessôa et al. 1972).

Lainson (1977) recorded haemogregarines in the erythrocytes of 46 of 60 (76.7%) young *Caiman c. crocodilus* (Linn. 1758) from Bragança, State of Pará, North Brazil, diagnosed as a species of *Hepatozoon* by its sporogonic cycle in experimentally infected *Culex fatigans.* No infections were seen in 14 juvenile specimens of another caiman, *Paleosuchus trigonatus*, but morphologically similar blood forms have been noted in the black caiman, *Melanosuchus niger*, from Pará (Lainson, unpublished observations).

In the present communication we report our studies on the life-cycle of *H. caimani* of *C. c. crocodilus*, involving the experimental infection of the mosquito *C. fatigans*, wild-caught and farmed frogs (*Leptodactylus fuscus* and *Rana catesbeiana*, respectively) and caimans that had been bred in captivity.

## MATERIALS AND METHODS

*Natural infection in the caimans, C. c. crocodilus and C. c. yacare* - Blood was obtained by clipping a claw or by heart puncture and thin smears air-dried, fixed in

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absolute methyl alcohol and stained by Giemsa's method. Two infected *C. c. crocodilus* showing abundant haemogregarines in the erythrocytes were sacrificed, and impression smears of liver, spleen, lungs and kidney stained by the same method. Pieces of these tissues were fixed in 10% buffered neutral formalin for histology. Similar material was obtained from six specimens of *C. c. yacare* from the State of Mato Grosso.

*Development in mosquitoes* - *C. fatigans* used in these experiments were from laboratory-bred colonies of mosquitoes originating from the outskirts of Belém, Pará, North Brazil: they were maintained at a temperature of from 24-26ºC. During the period 1992-1998 we fed a total of eight separate batches of these mosquitoes on restrained, infected caimans for the purpose of separate studies on the sporogonic cycle of *H. caimani* and experimental transmission to caimans and intermediate hosts.

For the sporogonic cycle, fully fed mosquitoes were dissected at 1, 2-3, 6, 9 and 12 h post feeding and the guts and contained blood clot smeared, fixed in methyl alcohol and stained by Giemsa's method. The remaining mosquitoes were periodically dissected in order to follow development of the oocysts in fresh coverslip preparations. For histology, some guts were fixed entire in 10% buffered neutral formalin, embedded in glycol metacrylate medium (GMA medium of Agar Scientific Ltd) and cut at 2-3 µm with a glass knife on a Sorval JB4 microtome: sections were stained with haematoxylin and eosin. Further material was fixed for transmission electron microscopy.

*Development in the frogs Leptodactylus fuscus and Rana catesbeiana* - In 1995, three wild-caught *L. fuscus* and three farm-bred *R. catesbeiana* were force-fed with batches of infected *C. fatigans* at 23 and 22 d.p.i. respectively, and in 1998 a further 10 *R. catesbeiana* were fed with other infected mosquitoes at 23 d.p.i. Some frogs were killed with chloroform at periods ranging from 14 to 28 d.p.i. and fresh, squash preparations of pieces of liver were examined under coverslips. Giemsa-stained dab smears were prepared from the liver, lungs, spleen and kidney. Some of the *R. catesbeiana* were retained for further observations and transmission experiments.

*Transmission to caimans* - Juvenile, uninfected caimans were obtained from the "Crocodile Safari" Zoological Gardens, on the outskirts of Belém, where they had been raised from eggs. They were maintained in an insect-screened animal house, on a diet of new-born laboratory white mice.

*Transmission via the frog R. catesbeiana* - Six of the frogs that had been fed with heavily infected mosquitoes in 1998 were sacrificed 30 d.p.i. and, following the detection of cysts in their livers, fed entire to six farm-bred caimans: blood films of these were periodically checked for the appearance of haemogregarines. Two of the caimans were killed 13 and 14 d.p.i., smears of liver, spleen, lung, kidney and the small intestine stained by Giemsa's method, and pieces of these tissues fixed in 10% buffered neutral formalin for histology. The surviving four animals were reserved for further observations.

*Transmission via infected mosquitoes* - Two farm-bred *C. c. crocodilus* were each force-fed with four *C. fatigans* from a batch of mosquitoes shown to have large numbers of mature oocysts at 23 d.p.i. They were retained for periodic examination of their blood for the appearance of gametocytes.

Photomicrographs were prepared using a Zeiss "Photomicroscope III" and Kodak TMX 100 film. All measurements are given in µm, followed by the range in parentheses.

## RESULTS

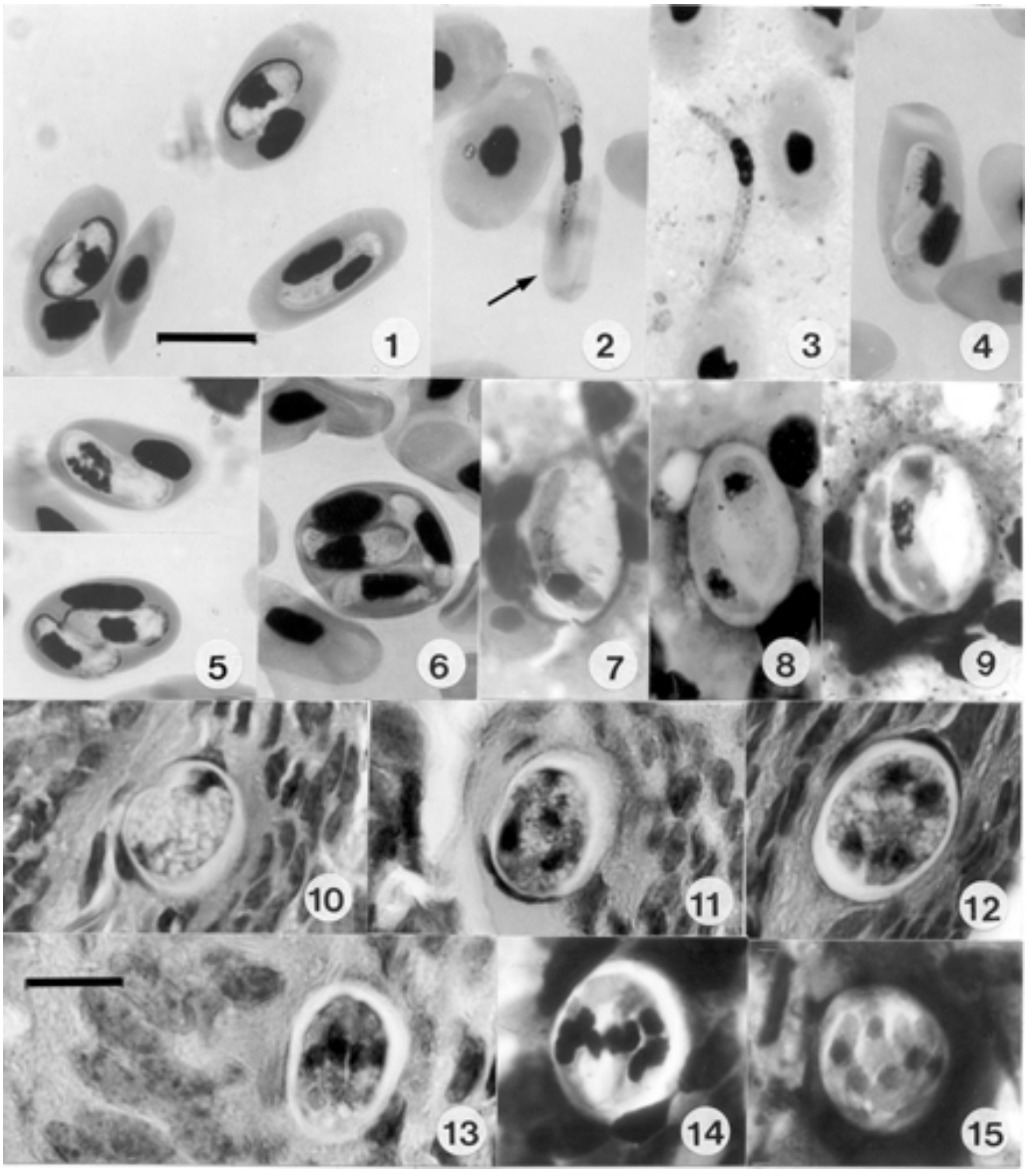
*Natural infection in the caiman, C. c. crocodilus and C. c. yacare* - Characteristic of the genus, the gametocytes of *H. caimani* show no sexual dimorphism and are restricted to the mature erythrocytes. They are enclosed in a capsule, which may or may not be strongly stained (Fig. 1), and from which occasional extracellular parasites can be seen to be emerging (Fig. 2). The larger intracellular gametocytes measure approximately 12.15 x 4.3 (10 x 3.75 – 13.75 x 3.75), (50 measured) and have a dense, intensely staining nucleus placed somewhat laterally in the parasite: less frequently it may be in the form of a widely dispersed reticulum (Fig. 5). Within their capsule the gametocytes are doubled up on themselves (Fig. 4) giving them a sausage-like appearance. Extracellular forms, however, appear as long, slim bodies, measuring 20.7 x 3 (16.2 x 2.5 – 25 x 4) (25 measured). They are only occasionally found in blood films (Figs 2, 3) and are best seen in the bloodmeal of mosquitoes recently fed on infected caimans (Fig. 16). Erythrocytes containing a single, mature gametocyte are rarely enlarged, but their nucleus is pushed to a lateral or polar position (Fig. 1): erythrocytes containing two, or even three parasites undergo some enlargement and deformation (Fig. 6).

No schizonts were detected in stained smears of the liver, spleen, lungs and kidney of the eight naturally infected caimans examined, but scanty to abundant monozoic and dizoic cysts were found in all of these tissues (Figs 7-9), predominantly in the liver. They measured 14.6 x 10 (12.5. x 6.25 – 21.5 x 21) (25 measured) and the contained zoites 12.5 x 3.7 (10 x 1.25 –15 x 4).

*Development in the mosquito* - Extra-erythrocytic gametocytes were readily detected in the smears of mosquito guts made from 1-12 h after these insects had fed on infected caimans (Fig. 16). In one smear made at 12 h the nucleus of a few elongate gametocytes, and other spherical forms, was divided into 2-4 portions (Figs 17, 18), but we were unable to detect stages typical of the adeleid association of male and female parasites, the production of gametes and the process of fertilization. A fresh coverslip preparation of a dissected mosquito gut made at 11 d.p.i. showed young, uninucleate sporonts under the elastic membrane on the outer surface of the midgut (Fig. 19), and Giemsa-stained smears made at 13 and 14 d.p.i. contained others with early nuclear division (Figs 20, 21). In fresh preparations made at this time there appeared the first signs of elevations on the surface of some parasites (Fig. 22), later to be thrown into the bulb-like protrusions into which the dividing nuclei migrate during formation of the sporocysts (Fig. 23).

Although developing sporozoites were seen in some sporocysts at 18 d.p.i, completely mature oocysts (Fig. 24) were first seen in dissected mosquitoes 21 d.p.i. and

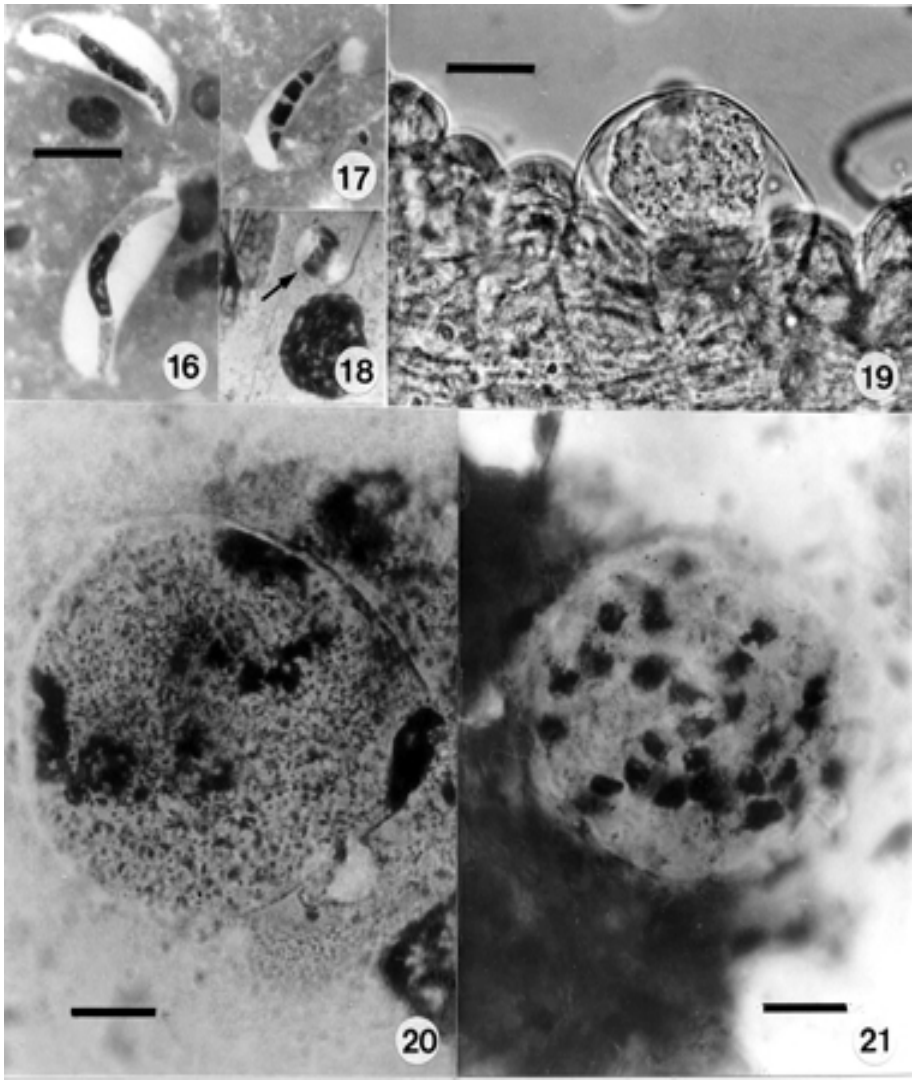
were crowded in large numbers in the haemocoele, on the surface of the intestine (Fig. 26). They measured up to 260 µm in diameter and possessed a delicate, colourless oocyst wall enclosed by the elevated elastic membrane of the midgut surface (Figs 24, 25). The largest seen contained an estimated 80-100 spherical sporocysts, but no apparent residuum. Sporocysts varied from 20-30 in diameter and contained an estimate of from 12-24 crescentic sporozoites budded off from a conspicuous residual body, rather like a hand of bananas (Figs 27, 28). Living sporo-




Figs 1-15. *Hepatozoon caimani* in *Caiman crocodilus crocodilus* and *C. c. yacare*. Fig.1: intraerythrocytic gametocytes: two showing conspicuous capsules and another apparently uncapsulated. Figs 2, 3: free gametocytes: one is emerging from its capsule (arrowed). Fig. 4: gametocyte, showing doubling up of the parasite in the erythrocyte. Figs 5, 6: multiple infection of erythrocytes, and a gametocyte with a reticulated nucleus. Figs 7-9: monozoic and dizoic cysts in liver smears. Figs 10-13: developing schizonts in the *lamina propria* of the ileum of two experimentally infected caimans, 13 and 14 days after these animals were fed with infected mosquitoes. Sections stained with haematoxylin and eosin. Figs 14,15: segmented schizonts, as seen in smears of the small intestine of the same animal, stained by Giemsa's method. Bars = 10 µm for all figures

zoites measured 19-22 x 4-5 (25 measured) and frequently showed conspicuous movements within the sporocyst: freed sporozoites fixed in Bouin's fluid and stained by a modified Giemsa's method measured slightly less, probably due to shrinkage following fixation (Figs 29, 30). The intensely staining nucleus is located more towards the broader and rounded extremity: large but less densely staining masses probably represent the crystalloid inclusions described in the sporozoites of *Hepatozoon* spp. by various authors (Smith & Desser 1998).

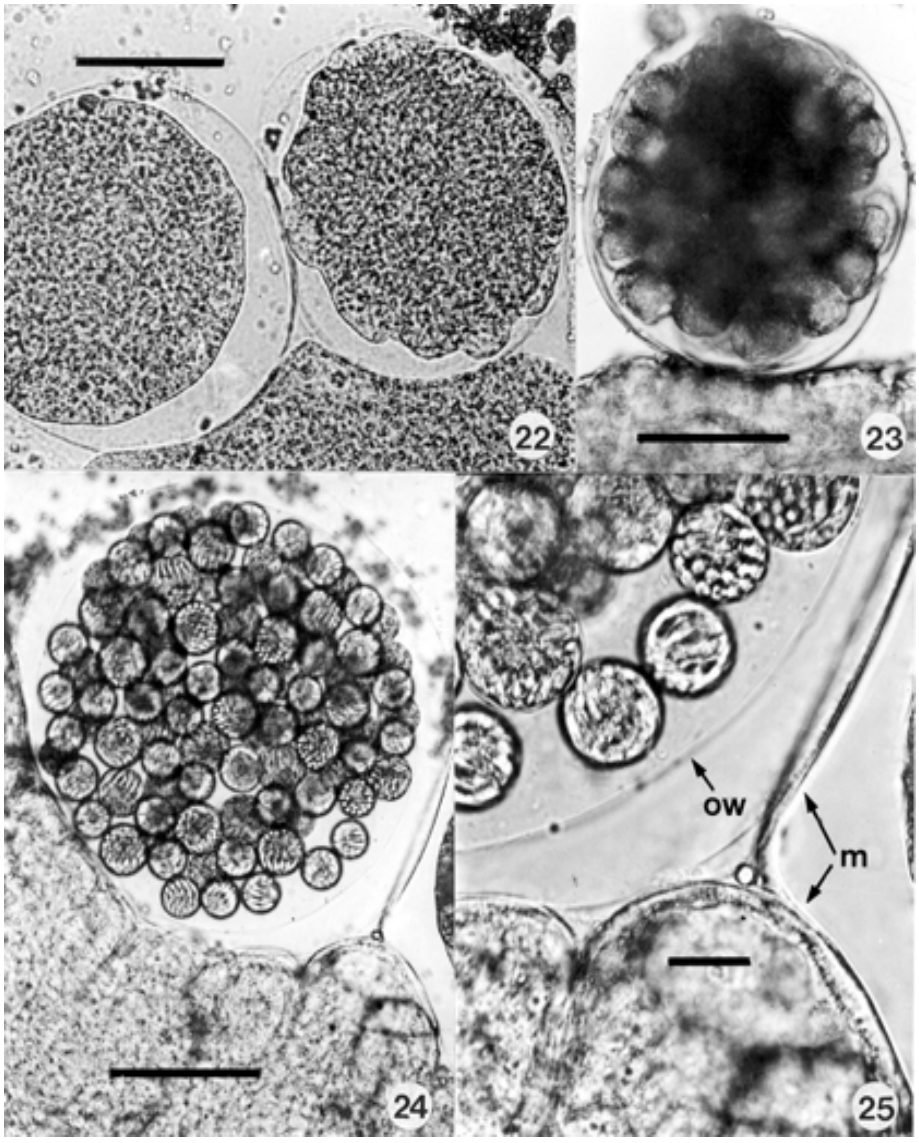
Both fresh preparations and sections of infected mosquitoes showed development to be remarkably asynchronous. Thus, at 13 d.p.i., single mosquitoes showed a mixture of parasites showing early and late sporoblast formation (Figs 31-34). In most cases sporocysts were fully mature at 21 d.p.i, but in some batches of mosquitoes the sporozoites were incompletely differentiated at 22 d.p.i, possibly the result of temperature fluctuations.




Figs 16-21: development of *Hepatozoon caimani* in the mosquito *Culex fatigans*. Fig. 16: freed gametocytes in a Giemsa-stained smear of the intestine and contained blood, 3 h after the blood-meal. Figs 17,18: elongated and spherical (arrowed) forms with divided nuclei in a smear made 12 h after the blood-meal. Fig. 19: freshly dissected mosquito gut, 11 days after the blood-meal, showing a uninucleate sporont beneath the elastic membrane on the outer surface of the midgut. Figs 20, 21: giemsa-stained smear of a mosquito gut and contents, 13 days after the blood-meal: early nuclear division of sporonts. Bars = 10 µm

*Development in the frogs Leptodactylus fusca and Rana catesbeiana* - Monozoic, dizoic and hexazoic cysts were abundant in the liver of *L. fuscus* 28 days after they had been fed with infected mosquitoes (Figs 35-37, 39).

No cysts were detected in the first three *R. catesbeiana* fed with infected mosquitoes at 22 d.p.i. in 1995: almost certainly this was due to the above-mentioned delay in differentiation of sporozoites, as shown in a parallel sample of infected mosquitoes subsequently examined by TEM. Cysts were consistently present, however, in the ten *C. catesebiana* fed with infected mosquitoes at 23 d.p.i. in 1998 (Figs 38, 40).



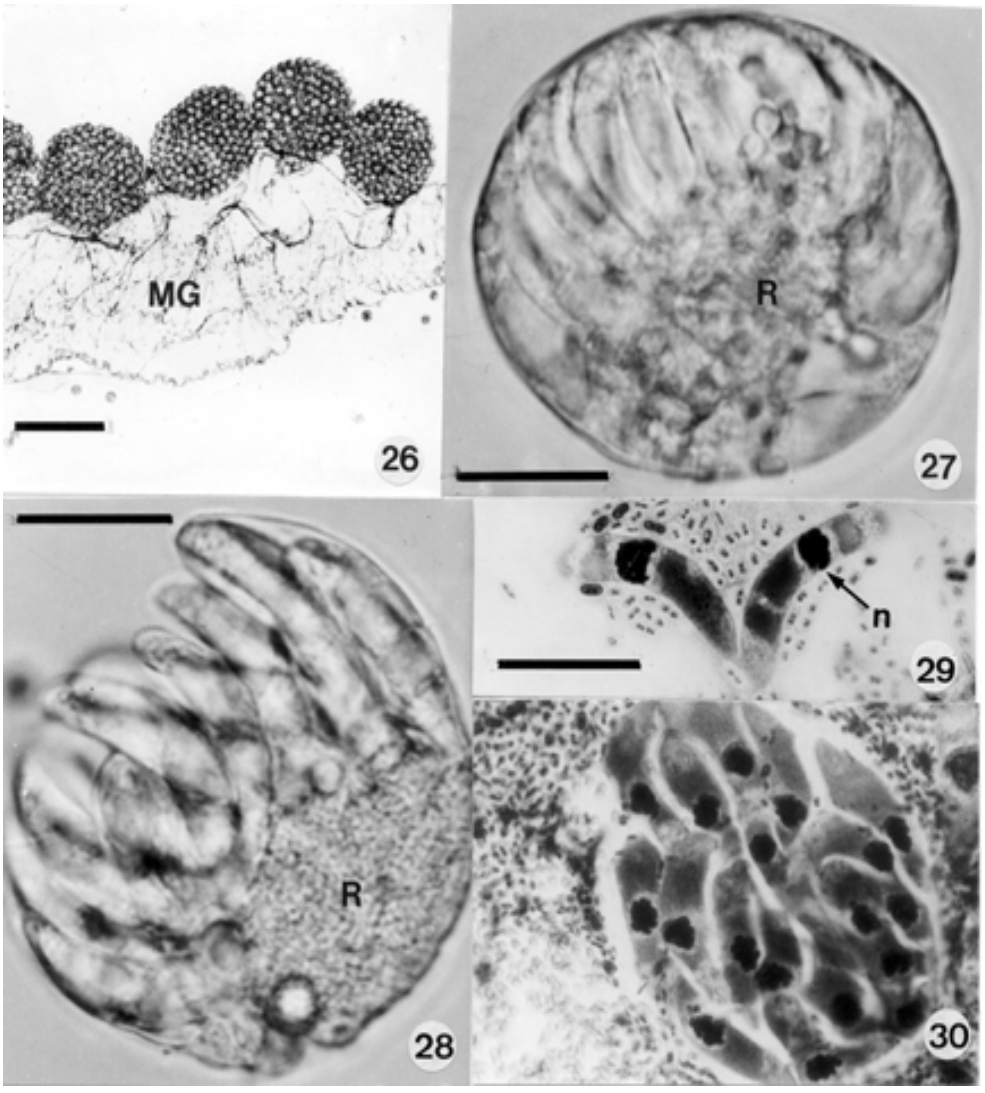

Figs 22-25: development of *Hepatozoon caimani* in *Culex fatigans*, as seen in coverslip preparations of freshly dissected mosquitoes. Figs 22, 23: developing elevations of the sporont surface prior to formation of the sporoblasts, 14 days after the blood-meal. Bar = 50 µm. Fig. 24: mature oocyst on the gut surface, 21 days after the infective blood-meal. Bar = 100 µm. Fig. 25: enlarged view of the same oocyst, showing its position beneath the stretched elastic membrane on the surface of the midgut (arrowed), ow: oocyst wall; m: membrane of the midgut surface. Bar = 20 µm

Development of the cysts, principally in the liver and less frequently in the lungs and the spleen, appeared to be within the reticulo-endothelial cells. Morphologically they were indistinguishable from those seen in the viscera of naturally infected caimans (Figs 7-9) . In fresh liver squashes (Figs 39-41) the cysts are ovoid to spherical bodies measuring 15 x 10 (14.5 x 12 – 21 x 20) (25 measured). They usually contained one or two slender zoites, very rarely four to six, and a prominant residual body of large spherules. In Giemsa-stained smears (Figs 35-38) the residual body was inapparent, possibly having been destroyed in the process of fixation.

No intraerythrocytic parasites were detected in any of the infected frogs.

*Transmission to caimans via cystic stages in the frogs L. fuscus and Rana catesbeiana* - One of the wild-caught frogs, *L. fuscus* showing abundant cysts in its liver, was fed to a young, wild-caught caiman in which no haemogregarines could be detected after repeated examination of stained blood films. Gametocytes were detected in its blood just over two months later.

The two *C. c. crocodilus* sacrificed 13 and 14 days after being fed with infected frogs showed no gametocytes in their blood, but developing and mature schizonts were abun-

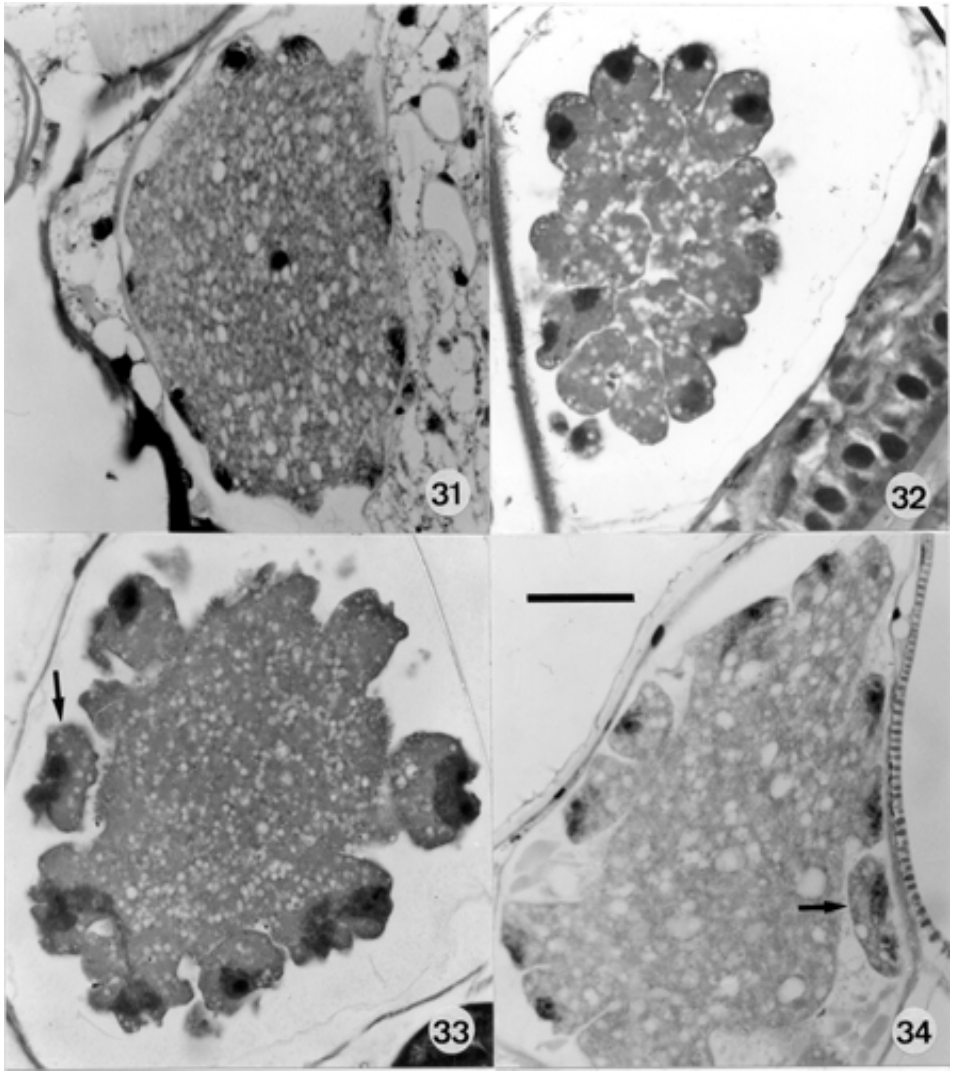



Figs 26-30: development of *Hepatozoon caimani* in *Culex fatigans.* Fig. 26: mature oocysts clustered on the gut surface, 21 days after the infective blood-meal. Fresh preparation. Bar = 200 µm. Figs 27, 28: freed, living sporocysts from a ruptured oocyst, showing sporozoites and prominent sporocystic residuum (R). Bars =10 µm. Figs 29, 30: freed, Giemsa-stained sporozoites from a ruptured sporocyst; n = nucleus. The cluster of sporozoites probably represents the entire contents of a single ruptured sporocyst. Bouin fixation. Bar = 10 µm

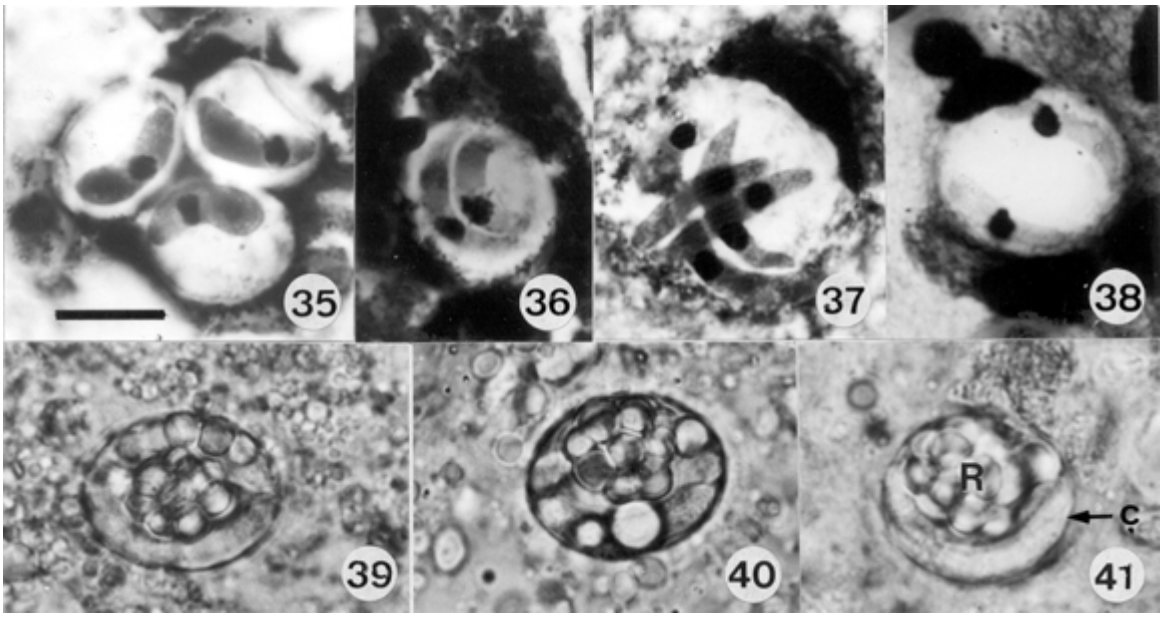
dant in the smears and sections of the *lamina propria* of the small intestine. Young undivided forms possessed a highly vacuolated cytoplasm containing from 2-6 nuclei (Figs 10-13). Segmented schizonts were 15.8 x 13 (13 x 9.6 – 20.7 x 18) as seen in Giemsa-stained smears (Figs 14, 15) and 16.5 x 12.2 (14 x 14 – 22.2 x 11.8) in sections (25 of each, measured). As far as we could ascertain they produced from 6 to 10 crescentic merozoites measuring approximately 11.2 x 2 (9.6 x 2.2 – 16 x 2.2): the number may have been greater in some schizonts in which some nuclei were superimposed on others. No schizonts were detected in the liver, spleen, lungs and kidneys of these animals.

The four surviving caimans fed with infected frogs showed no gametocytes in their blood when examined 52 days later, but gametocytes were present in their erythrocytes when they were next examined 79 d.p.i. The prepatent period was, therefore, at some time between 52-79 days.

*Transmission to caimans via infected mosquitoes* - The two *C. c. crocodilus* fed with infected mosquitoes




Figs 31-34: asynchronous sporogonic development of *Hepatozoon caimani* in a single *Culex fatigans*, 13 days after the blood-meal. Fig. 31: beginning of sporoblast formation. Fig. 32: more advanced stage, with uninucleate sporoblasts almost budded off. Figs 33, 34: separated (arrowed) and separating sporoblasts, with early nuclear division subsequently giving rise to the sporocysts and contained sporozoites. Histological sections stained with haematoxylin and eosin. Bar = 10 µm for all figures




Figs 35-41: resting cystic stages of *Hepatozoon caimani* in a variety of experimental secondary vertebrate hosts. Figs 35-37: in Giemsa-stained liver smears of the frog *Leptodactylus fuscus*: monozoic, dizoic and hexazoic cysts (one cystozoite in the latter is in a different focal plane). Fig. 38: in the frog *Rana catesbeiana.* Figs 39-41: living cysts in squash preparations of liver from the frogs *L. fuscus* and *R. catesbeiana* and the lizard *Neusticurus bicarinata,* following their ingestion of infected *Culex fatigans;* c: cystozoite; R: residual body. Bar =10 µm for all figures

first showed gametocytes in their peripheral blood 82 days later, the prepatent period being somewhere between 52-82 days. Monozoic and dizoic cysts, indistinguishable from those developing in frogs and in the viscera of naturally infected caimans, were relatively abundant in smears of the liver, but rare in those of the spleen, lungs and kidneys. No schizogonic stages were detected.

## DISCUSSION

It remains highly probable that most haemogregarines described in crocodilians throughout the world are species of *Hepatozoon.* We feel, however, that it is unwise to transfer all of those described under the name of *Haemogregarina* to the genus *Hepatozoon* until observations have been made on their sporogonic cycle in the invertebrate host. Thus, allocation of the name *Haemogregarina crocodilinorum* to a haemogregarine of the American alligator by Börner (1901) may have been justified, for Khan et al. (1980) have since described erythrocytic schizogony of a haemogregarine in this crocodilian, and a sporogonic cycle typical of the genus *Haemogregarina* in leeches removed from wild-caught alligators.

From their very similar morphology and the pattern of their sporogonic cycles, we consider the haemogregarines of *C. latirostris* and *C. crocodilus* to be conspecific, namely *H.caimani* (Carini 1909, Pessôa et al. 1972). The haemogregarine of another genus, *Melanosuchus niger*, has indistinguishable blood forms and a similar sporogonic cycle (Lainson, unpublished observations), which leads us to suggest that all the *Hepatozoon* species of the Alligatorinae (American caimans and alligators) may, in fact, be *H. caimani.* Cross-infection experiments and DNA analyses are needed to settle the question as to just how many valid species of the genus exist in crocodilians of both the Old World and the Americas.

In discussing the transmission of *H. pettiti* of *Crocodylus niloticus*, Hoare (1932) suggested that this occurred when an infected tsetse fly settles in the open mouth of the crocodile to feed and when the animal, irritated by the bite, "... may snap its jaws and crush the fly, thus liberating the cysts of the haemogregarine in the buccal cavity". We succeeded in transmitting *H. caimani* to clean caimans by feeding them with infected *C. fatigans* and, as newly hatched caimans snap at almost everything that moves, it quite likely includes mosquitoes coming to feed on them. It is notable that many of the infected animals we studied were estimated to be only a few months old.

Landau et al. (1970a, 1970b, 1972) indicated the important role of endogenous cysts located in the tissues of a secondary host in the transmission of *Hepatozoon* species. In a study of *H. domerguei* of snakes and lizards in Madagascar they described the life-cycle as follows: mature oocysts develop in mosquitoes which have ingested gametocytes during a blood-meal on an infected snake, and the mosquitoes may then be eaten by the lizard host, allowing the released sporozoites to gain entrance into the viscera, principally the liver. Here they become encysted and, by the process of successive endodyogenies, produce from two to six cystozoites. The cysts remain latent in the tissues until the lizard is eaten by the snake predator, when the cystozoites are released and penetrate organs, such as the liver and lungs. In these organs they undergo successive, large-progeny

schizogonic divisions which eventually complete the cycle by the production of gametocytes which invade the peripheral blood. From our present study we feel that a similar process is the predominant mode of transmission for *H. caimani.*

Whether or not frogs are the major source of cysts of this parasite in nature remains to be determined. During the present studies infected mosquitoes were also fed to specimens of the semi-aquatic teiid lizard *Neusticurus bicarinatus* and cysts containing from 1-4 cystozoites were later encountered in the liver and lungs of these animals (Fig. 41). Unfortunately, it is uncertain if these cysts were those of *H. caimani* or of another *Hepatozoon* we have occasionally encountered in the erythrocytes of *N. bicarinatus*, and the sporogony of which has also been followed in *C. fatigans* (Lainson, unpublished observations).

The number of cystozoites produced per cyst seems to vary both with the species of *Hepatozoon* and within the same species. Monozoic cysts have been recorded for *H. griseisciuri* of squirrels (Desser 1990); both monozoic and dizoic for *H. balfouri* of jerboas (Hoogstraal 1961); dizoic for *H. sauromali* of the iguanid lizard *Sauromalus hispidus* (Lewis & Wagner 1964), monozoic to hexazoic for *H. domerguei* (Landau et al. 1972) and *H. caimani* (present investigation), and dizoic to octozoic for *H. kisrae* of the lizard *Agama stellio* (Paperna et al. 2002). Cysts containing four or more cystozoites may present problems in their differentiation from mature, primary schizonts, which are of similar size. Prior to maturity, however, the schizonts may be recognised by the presence of several nuclei in the undivided cytoplasm (Figs10-12), whereas in the cysts successive endodyogenies immediately result in a pair, or pairs, of cystozoites. In addition, both sporozoites and cystozoites usually contain prominant crystaloid inclusions (Smith & Desser 1998), which are absent in merozoites. In the present study, although such inclusions were very conspicuous in Giemsa-stained sporozoites from ruptured oocysts (Fig. 29), they were much less obvious in cystozoites in the tissues of the frogs and caimans (Figs 7-9, 35-38), and Paperna et al. (2002) noted their apparent absence in some cystozoites of *H. kisrae.*

A surprise in our study has been the detection of schizonts only in the *lamina propria* of two experimentally infected caimans sacrificed at 13 and 14 d.p.i., and our inability to demonstrate schizogonic stages in the liver, lungs, spleen and kidney of eight animals with natural infections of undetermined duration. This, and the failure of other authors to demonstrate schizonts in the viscera of naturally infected caimans (Carini, 1909, Di Primio 1925, Pessôa et al. 1972) suggests that after the penetration of the intestinal epithelium by sporozoites or cystozoites, subsequent schizogony is limited to the *lamina propria* of the small intestine. It is unfortunate that, in the expectancy that schizonts would be located in the liver, spleen or kidney, we did not examine the intestines of the naturally infected animals. In other species of *Hepatozoon*, particularly those of snakes, large schizonts, producing many merozoites, are usually abundant in the liver, lungs and other organs.

Both the natural invertebrate vector(s) and the intermediate vertebrate host(s) of *H. caimani* remain to be determined. It may be that a variety of haematophagous arthropods can serve as vectors, for the full sporogony of *Hepatozoon* spp. has been recorded in experimental or natural infections of *C. fatigans, C. tarsalis, C. pipiens* and *C. territans* (Mackerras 1962, Booden et al. 1970, Bashtar et al. 1984, Desser et al. 1995), *Aedes togoi* and *Aedes aegypti* (Ball et al. 1969, Lowichik et al. 1993), *Anopheles stephensi* (Landau et al. 1972), the ticks *Argas brumpti* and *Hyalomma aegyptium* (Garnham 1955, Paperna et al. 2002), triatomid bugs (Da Rocha e Silva 1975), a sand fly, *Lutzomyia* sp. (Lainson, unpublished observation) and the tsetse fly, *Glossina palpalis* (Hoare 1932). We have found *H. caimani* to develop equally well in *C. fatigans* and *A. aegypti*, both of which feed avidly on caimans in the laboratory. Finally, in view of the role of tsetse flies in the transmission of *H. pettiti* in Africa, tabanids must figure in the list of suspects, especially as four different species of these flies have been shown to feed on *C. c. crocodilus* in Amazonian Brazil (Ferreira et al. 2002). There have been a number of unsuccessful attempts to transmit haemogregarines of both crocodilians and snakes by leeches, although the parasites have on occasions produced sporulated oocysts (Pessôa & Cavalheiro 1969a, b, Khan et al. 1980, Ball 1958, Smith et al. 1994). These, and our own failure to obtain development of *H. caimani* in leeches suggests them to be unsuitable vectors of *Hepatozoon.*

In their description of the development of *H. caimani* in the mosquito *Culex dolosus*, Pessôa et al. (1972) recorded the apparent division of the sporont of young oocysts into two "sporoblasts", one of which degenerated while the other completed development in the usual way. We failed to see such division of the sporont and are of the opinion that the two bodies they observed represented two sporonts in close apposition and enclosed by the overlying elastic membrane of the insect stomach. The individual oocyst walls of the two parasites are clearly visible in the Figs 6 and 7 of these authors.

Landau et al. (1972) showed that different genera of lizards and snakes can harbour cysts of *H. domerguei*, and that gametocytes of this parasite circulate in the peripheral blood of both the snake and the lizard hosts, thus greatly facilitating infection of the mosquito vector. Our demonstration of resting cysts of *H. caimani* in two different genera of frogs, *Leptodactylus* and *Rana* suggests that, in the same way, there may be a wide range of anuran hosts for *H. caimani.* As far as we are aware there was no development of gametocytes of this parasite in the blood of the experimental frogs. Although Paperna and Smallridge (2001) found that gametocytes of *Hemolivia mariae*, a haemogregarine of the Australian lizard *Tiliqua rugosa*, eventually did appear in the blood of lizards of other genera that had been fed with infected tick viscera, it was only after an abnormally long prepatent period. Quite likely, the more drastic move of *H. caimani* from a reptilian host to an amphibian may entirely preclude the production of gametocytes in frogs.

The possible role of lizards as secondary hosts of the cystic stages of *H. caimani* requires further investiga-

tion, and our apparent transmission of this parasite to the lizard *Neusticurus bicarinatus* needs confirmation with laboratory-bred lizards.

As cysts containing cystozoites were readily demonstrable in the tissues of both naturally and experimentally infected caimans, a third route of transmission by cannibalism needs to be considered. Cannibalism appears to be most frequent among juvenile crocodilians of different size in overcrowded conditions (Alderton 1991).

There remain other gaps in our knowledge of the life cycle, in particular the fertilization process in the invertebrate vector. This probably follows a similar pattern to that described by Mackerras (1962) for *H. breinli* of the Australian lizard *Varanus tristis* in experimentally infected *C. fatigans*; namely, association of the male and female gametocytes, production of four flagellated gametes by each microgametocyte, fertilization and the production of the zygotes giving rise to the oocysts. Landau et al. (1972), however, described the microgametocyte of *H. domerguei* as producing only two gametes. Possibly, our failure to find undoubted stages of the fertilization process was because a search for them was not made beyond 12 h p.i.

If one accepts the hypothesis of co-speciation, which postulates that parasites and their hosts speciate in synchrony (Brooks 1979), the apparent restriction of the schizogony of *H. caimani* to the *lamina propria* of the small intestine is of particular interest. It suggests that invasion of the liver and other organs in the more evolved vertebrate hosts may have been of secondary development in the evolution of the genus *Hepatozoon.*

A further paper is to be published on the ultrastructure of the sporogonic stages of *H. caimani*.

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