ULTRASTRUCTURAL FEATURES OF THE GAMETOGENIC AND SPOROGONIC DEVELOPMENT OF *HEPATOZOON SIPEDON* (APICOMPLEXA: ADELEORINA)

The applicability of ultrastructural data in differentiating among *Hepatozoon* species

SMITH T.G.* & DESSER S.S.*

Summary :

Stages of gametogony and sporogony of the haemogregarine *Hepatozoon sipedon*, an apicomplexan parasite of the Northern water snake, *Nerodia sipedon sipedon*, were studied in the mosquito, *Culex pipiens*, by electron microscopy. Four days after mosquitoes fed on an infected snake, microgamonts and macrogamonts were observed in syzygy in parasitophorous vacuoles within fat body cells of the haemocoel. During microgametogenesis, two biflagellated microgametes were formed, one of which fertilized the macrogamete. After fertilization, zygotes increased rapidly in size, accumulating reserve material in the form of lipid inclusions. Beginning at 16 days post-feeding (PF), the nucleus of the immature oocyst underwent multiple divisions in the first stage of sporoblast formation. Small crystalloid bodies initially appeared in the cytoplasm of the dividing oocyst at 20 days PF. At 24 days PF, the oocyst contained hundreds of sporoblasts, each of which matured as early as 28 days PF into thick-walled sporocysts containing eight sporozoites and a large residual body. Sporozoites contained a large crystalloid body comprised of tightly-packed particles assembled in a paracrystalline array. The use of ultrastructural characters in the differentiation of *Hepatozoon* species is discussed in context with the current phylogenetic hypotheses of adeleorin taxa.

KEY WORDS : *Culex pipiens*, gametogony, haemogregarine, *Hepatozoon*, mosquito, sporogony, ultrastructure.

Résumé : CARACTÉRISTIQUES ULTRASTRUCTURALES DU DÉVELOPPEMENT GAMÉTOGÉNIQUE ET SPOROGONIQUE D'*HEPATOZOON SIPEDON* (APICOMPLEXA: ADELEORINA) : Applicabilité des caractéristiques ultrastructurales pour la séparation des espèces d'*Hepatozoon*

Les stades de la gamétogonie et de la sporogonie de l'hémogrégarine Hepatozoon sipedon, *un parasite du phylum Apicomplexa qui infeste la Couleuvre d'eau* (Nerodia sipedon sipedon), *ont été étudiés dans le moustique* Culex pipiens *à l'aide de la microscopie électronique. Quatre jours après que le moustique se soit nourri sur une couleuvre, un microgamonte et un macrogamonte se sont associés (syzygyie) dans une vacuole parasitophore à l'intérieur de l'hémocoèle du moustique. La microgamétogénèse a produit deux microgamètes bi-flagellés, dont un a fertilisé la macrogamète. Après la fertilisation, les zygotes ont grandi rapidement et amassé d'importantes réserves sous forme d'inclusions lipidiques. Après le 16e jour, le noyau du jeune oocyste a subi des divisions multiples au cours du premier stade de la formation des sporoblastes. Les cristalloïdes apparaissent dans le cytoplasme de l'oocyste après le 20e jour. Le 24e jour, l'oocyste contient de nombreux sporoblastes, qui murissent tous après le 28e jour dans des sporocystes qui contiennent huit sporozoïtes et un grand corps résiduel. Les sporozoïtes contiennent un grand cristalloïde composé de petites particules en arrangement paracristallin. L'usage des caractéristiques ultrastructurales pour la séparation des espèces d'*Hepatozoon *est présenté dans le contexte des hypothèses sur les phylogénies courantes des genres du sous-ordre Adeleorina.*

MOTS CLÉS : Culex pipiens, *gamétogonie, hémogrégarine,* Hepatozoon, *moustique, sporogonie, ultrastructure.*

INTRODUCTION

Species of the genus *Hepatozoon* are haemogregarine parasites characterized by gametogenic and sporogonic development, with the formation of large multisporocystic oocysts, in an arthropod definitive host, and merogonic and gamontogonic development in the internal organs and blood cells of a vertebrate intermediate host after an infected arthropod is ingested (Levine, 1988). Over 300 species of *Hepatozoon* have been reported from all groups of tetrapod vertebrates, although over 90 % of these have been described solely on the basis of bloodstream gamonts (Smith, 1996). The morphological features of intraerythrocytic and intraleucocytic stages are insufficient to separate members of this genus from those of other genera of haemogregarines infecting terrestrial vertebrates, namely *Haemogregarina, Hemolivia* & *Karyolysus* (Mohammed & Mansour, 1959; Ball *et al.*, 1967), let alone to confer specific status.

The most useful criteria for the generic placement of haemogregarines and the differentiation of *Hepatozoon* species are features of gametogony and sporogony in the invertebrate host (Ball, 1967), which for members of this genus may be either a tick, mite, louse, dipteran, or flea (Telford, 1984). However, with the excep-

*Department of Zoology, University of Toronto, Toronto, Ontario, Canada M5S 3G5.
Correspondence: Sherwin S. Desser. Tel: (416) 978-6956 – Fax : (416) 978-8532 – E-mail <wired@zoo.toronto.edu>.

tion of a few ultrastructural studies (Vivier *et al.*, 1972; Göbel & Krampitz, 1982; Bashtar *et al.*, 1984*b*; Lowichik *et al.*, 1993; Desser *et al.*, 1995), data for sexual development are often incomplete or restricted to stages observed by light microscopy. In this study, we describe the ultrastructure of the gametogenic and sporogonic stages of *Hepatozoon sipedon,* a species infecting Northern water snakes in *Culex pipiens*, a potentially natural definitive host (Smith *et al.*, 1994).

MATERIALS AND METHODS

Collection and maintenance of experimental animals

Northern water snakes *(Nerodia sipedon sipedon)* were collected from marshes near the Queen's University Biological Station (44° 35′ N; 76° 15′ W), north of Kingston, Ontario, Canada in May, 1992. Smears made of blood collected from the caudal vein were fixed and stained with Diff-Quik®, and examined for haemogregarines. Snakes were housed in mesh-covered terraria and maintained on a diet of chopped worms and fish.

Larvae of the mosquito *Culex pipiens*, obtained from Carolina Biological Supply Company, were fed a mixture of TetraMin® fishfood and yeast, and maintained in dechlorinated tap water at 25 °C in shallow plastic tubs. Pupae in small jars of water were transferred to screened plastic boxes. Adults were provided with distilled water and a 10 % sucrose solution, and maintained at 25 °C at 75-80 % humidity on a 14:10 hour day/night cycle.

Examination of developmental stages

Mosquitoes deprived of water and sucrose for 12 and 36 hours, respectively, were allowed to feed on an unrestrained infected water snake in a glass feeding cage in the dark at 25 °C. Engorged mosquitoes were maintained as described above.

Wet mounts of the abdominal contents of *C. pipiens* were examined at 28 days PF. Single oocysts were ruptured by applying gentle pressure on an overlying cover slip in order to observe and count sporocysts.

Abdomens of blood-fed *C. pipiens* were dissected at 1, 4-14, 16, 18, 20, 22, 24 and 28 days PF and fixed in 2.5 % (v/v) glutaraldehyde in 0,09 M Sörensen's phosphate buffer (Bozzola & Russell, 1992), pH 7.1, for 72 hours. Specimens were post-fixed in 4 % osmium tetroxide in 0.13 M Sörensen's buffer containing 0.8 % (w/v) potassium ferrocyanide and 0.15 M sucrose, dehydrated through an ascending graded ethanol series, and infiltrated and embedded in Spurr's resin (Spurr, 1969). Semithin sections (0.5 µm), cut using a Reichert Ultracut-E ultramicrotome, were stained with 1.0 % toluidine blue and post-stained with 1.0 % basic fuchsin. Ultrathin sections were stained using 2.0 % (w/v) uranyl acetate in 50 % methanol, post-stained in Reynolds' lead citrate (Reynolds, 1963), and examined with a Hitachi H7000 electron microscope.

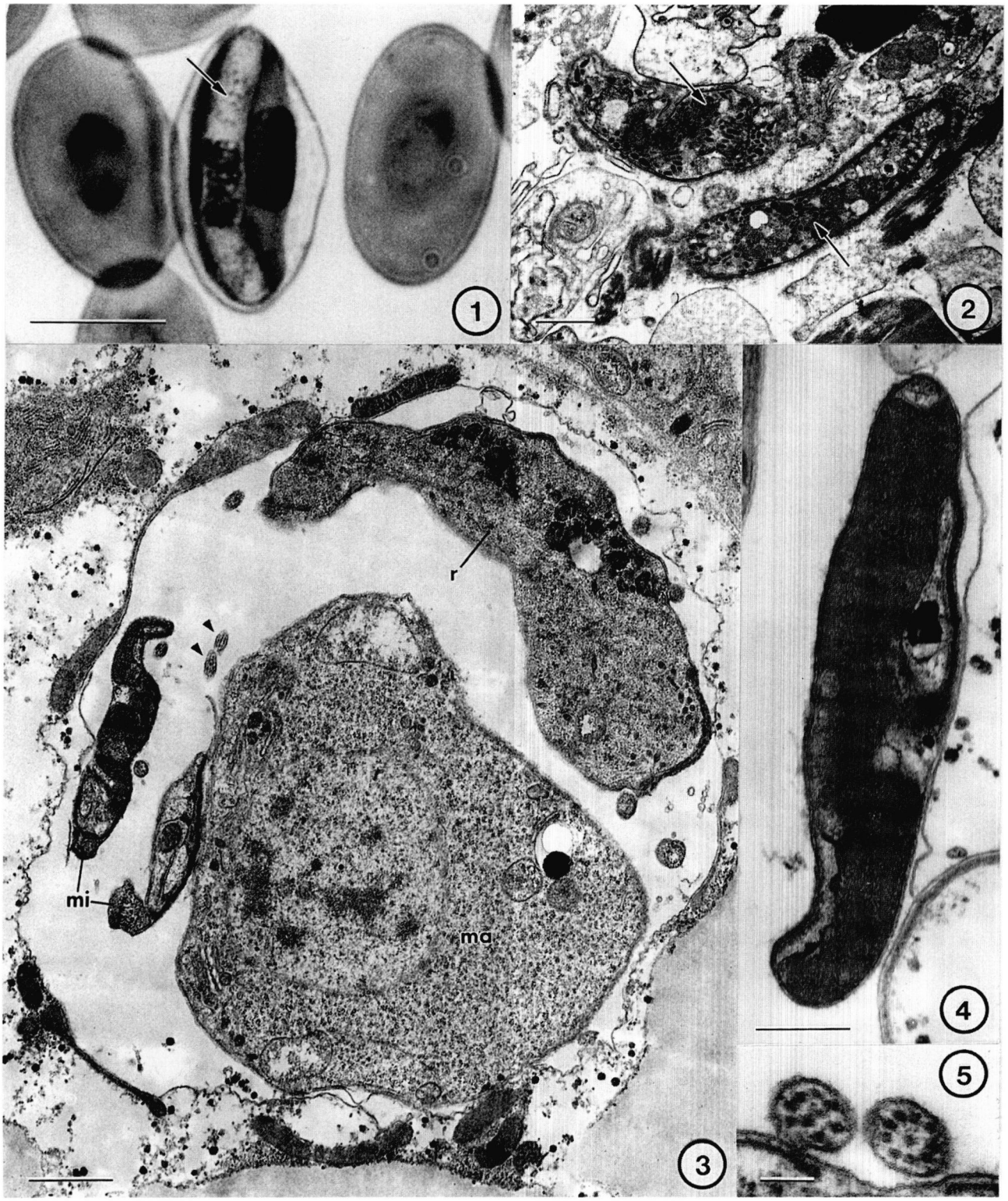
All photomicrographs, including those of wet mounts and semithin sections, were taken with a Zeiss Universal 1 photomicroscope using Kodak T-MAX 100 film.

RESULTS

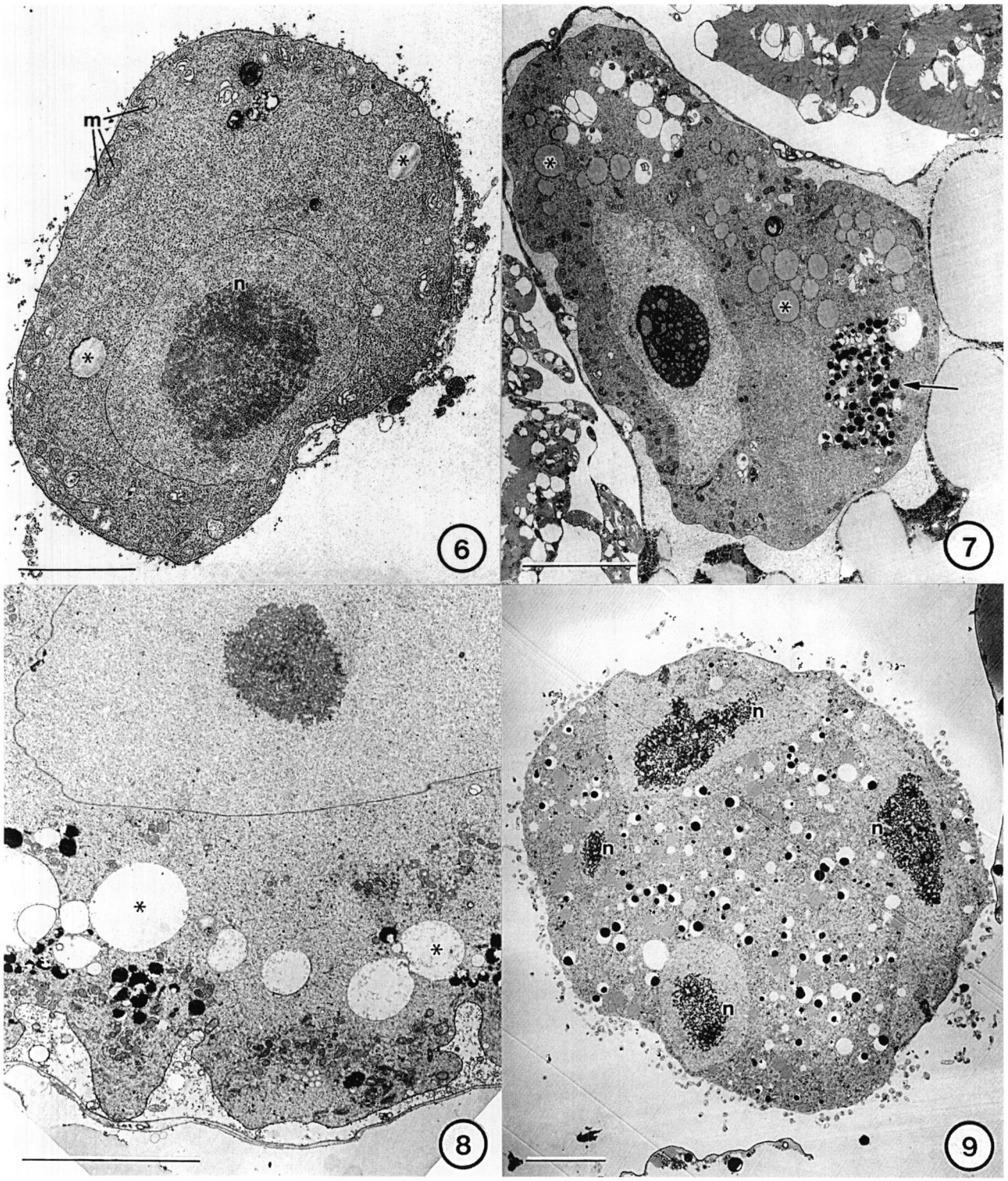
At 1 day PF, gamonts of *Hepatozoon sipedon*, which maintained the elongated structure observed in blood films (Fig. 1), had emerged from ingested erythrocytes and were observed in groups of two, three or four (Fig. 2) among blood cells in the gut of the mosquito.

At 4 days PF, gamonts that had paired in syzygy within a parasitophorous vacuole were observed undergoing gametogenesis within the cytoplasm of fat body cells in the haemocoel of the mosquito *C. pipiens* (Fig. 3). The macrogamete contained large mitochondria and a nucleus that appeared similar to that of a gamont. At the completion of microgametogenesis, microgametes were observed adjacent to the large macrogamete and the residual body of the microgamont, an irregular structure still containing micronemes and the remains of an apical complex (Fig. 3). Examination of serial semithin sections revealed that two biflagellate microgametes were formed from each microgamont. Microgametes consisted almost completely of an electron-dense nucleus (Fig. 4), surrounded by a typical trilaminar pellicle. Cross-sections through the flagella of the microgametes typically revealed a 9 + 1 arrangement of singlet microtubules within a trilaminar flagellar sheath (Fig. 5).

Sporogonic development was observed between 5 and 28 days PF. Zygotes, each contained within a parasitophorous vacuole, rapidly increased in size after fertilization. At 6 days PF, immature oocysts contained numerous mitochondria around the periphery of the cell and a few dispersed lipid inclusions (Fig. 6). The nucleus contained a granular nucleolus which was considerably more condensed than that observed in macrogametes. A substantial increase in the number of lipid inclusions and electron-dense vesicles was evident in immature oocysts at 13 days PF (Fig. 7). The nucleus at this stage was proportionally smaller, with a highly-condensed nucleolus. The intracellular nature of sporogonic development was still apparent at this



Figs. 1-5. — Gametogenesis of *Hepatozoon sipedon*. 1. Light micrograph of gamont (arrow) in erythrocyte of Northern water snake. Bar = 10 µm. 2. Two gamonts (arrows) that have emerged from erythrocytes in the gut of *Culex pipiens* at 1 day post-feeding (PF). Bar = 1 µm. 3. A macrogamete (ma) with diffuse chromatin, two microgametes (mi), and the residual body of the microgamont (*r*) in a fat body cell at 4 days PF. Cross-sections through the flagella of the microgametes are indicated by arrowheads. Bar = 1 µm. 4. Microgamete at higher magnification, revealing the large electron-dense nucleus (asterisk) that occupies most of the cell. Bar = 0.5 µm. 5. A higher magnification of a cross-section through the flagella of the microgamete, showing the 9 + 1 arrangement of singlet microtubules within a trilaminar flagellar sheath. Bar = 0.1 µm.



Figs. 6-9. — Early sporogonic development of *H. sipedon*. 6. Immature oocyst at 6 days PF, revealing the nucleus (*n*), peripherally-arranged mitochondria (*m*) and a few dispersed lipid inclusions (asterisks). Bar = 5 µm. 7. Immature oocyst at 13 days PF, showing an increase in the number of lipid inclusions (asterisks) and the appearance of electron-dense vesicles (arrow). Note the intracellular aspect of the developing oocyst. Bar = 10 µm. 8. Immature oocyst at 14 days PF, with invaginations of the cytoplasmic membrane. Lipid inclusions (asterisks) and electron-dense vesicles are numerous. Bar = 10 µm. 9. Immature oocyst at 16 days PF, revealing four nuclei (*n*), each with a diffuse nucleolus. The electron-dense vesicles are most numerous at this stage. Bar = 10 µm.

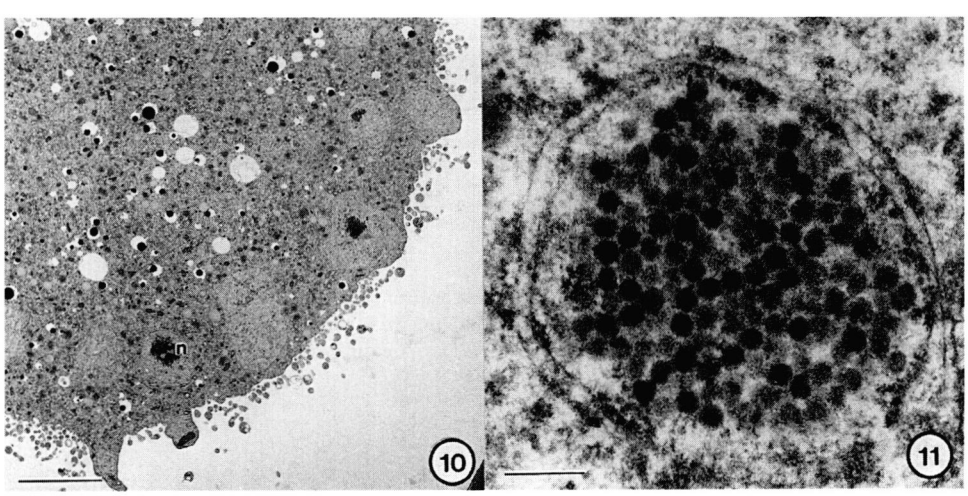
stage, although due to the increasing size of the immature oocyst, it was not obvious later in development. At 14 days PF, the cytoplasmic membrane of the immature oocyst began to invaginate (Fig. 8). Many small mitochondria occurred in the cytoplasm of the oocyst between these invaginations. Serial sections revealed that the nucleus of the oocyst had divided into four smaller nuclei at 16 days PF (Fig. 9). Other features included many lipid inclusions smaller in size than those seen at earlier stages, small electron-dense vesicles, and numerous small blebs of cytoplasm extending from the oocyst. Each nucleus contained a diffuse granular nucleolus and regions of heterochromatin. At 20 days PF, the nuclei of the oocyst had subdivided further and were found near the periphery of the oocyst (Fig. 10). In addition to abundant mitochondria and lipid inclusions, the cytoplasm of the developing oocyst contained small crystalloid bodies consisting of clumps of round particles surrounded by rough endoplasmic reticulum (Fig. 11). A decrease in the number of electron-dense inclusions was concomitant with the increase in the number of small crystalloid bodies.

By 24 days PF, numerous sporoblasts (Fig. 12) were found within developing oocysts. Developing sporoblasts, each invested by a trilaminar pellicle, included large crystalloid bodies, amylopectin and lipid inclusions, and one or more nuclei that were often observed near the periphery of the sporoblast. Crystalloid bodies in sporoblasts were organized into paracrystalline arrays (Fig. 13) and lacked the enclosing rough endoplasmic reticulum seen in immature oocysts. Sporoblast nuclei contained a dense nucleolus, unlike the granular nature observed in earlier stages. Other distinctive features of sporoblasts included large projections of cytoplasm (Fig. 14), and the presence of micropores on the sporoblast surface (Fig. 14, inset).

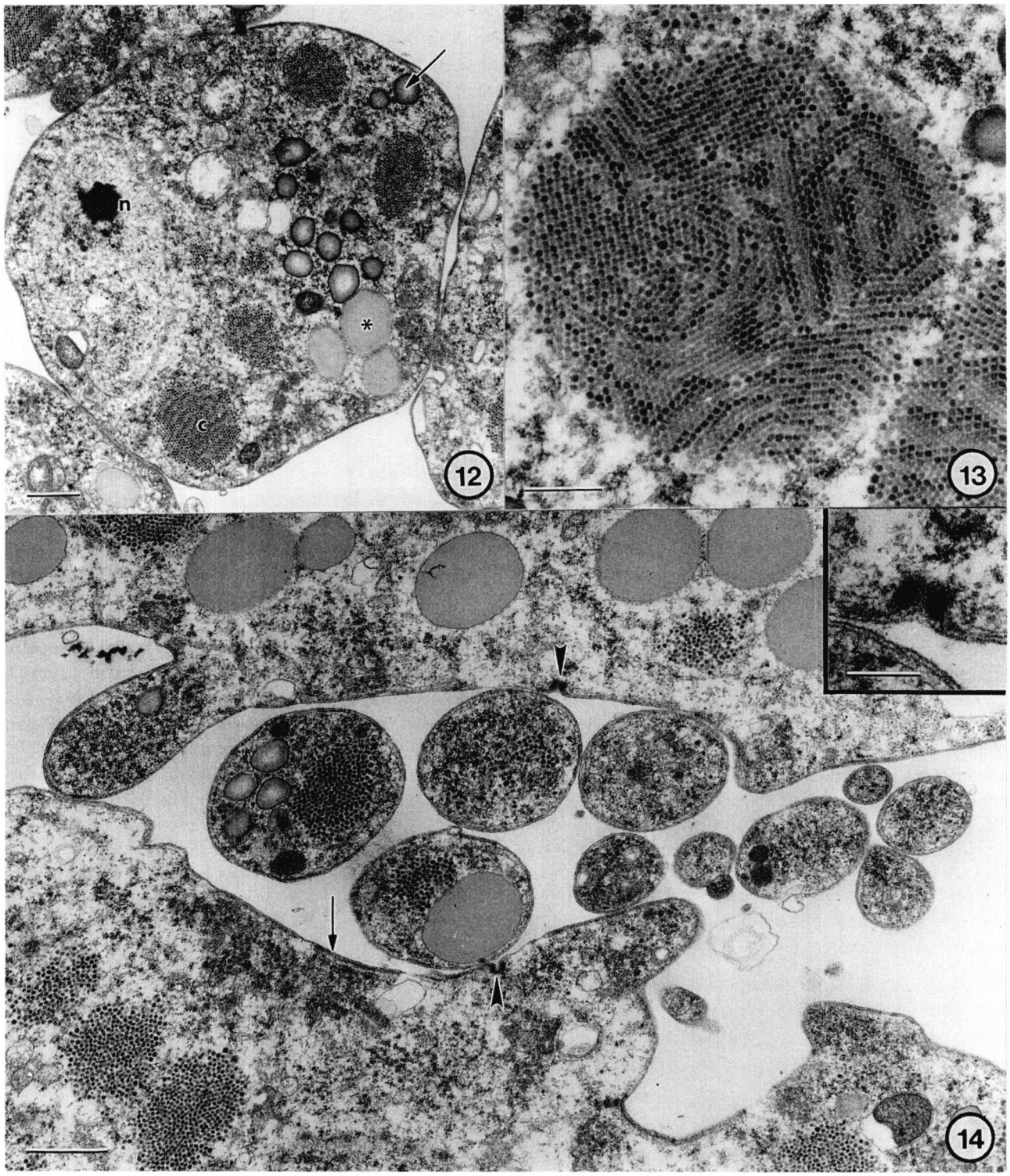
By 28 days PF, most oocysts were mature, consisting of hundreds (mean = 591, n = 5) of differentiated sporocysts enclosing fully-formed sporozoites. Mature oocysts (Fig. 15) were spherical and averaged 262.6 ± 23.0 µm (n = 20) in diameter; mature sporocysts (Fig. 16) were spherical and averaged 18.2 ± 1.0 µm (n = 40) in diameter. Individual sporocysts (Fig. 17) contained eight sporozoites and a large residual body enclosed within a thick sporocyst wall composed of two layers (Fig. 17, inset A). The residual body, which enclosed large amylopectin and lipid inclusions, was bounded by a single membrane, in contrast to the trilaminar pellicle surrounding the sporozoites (Fig. 17, inset B). Sporozoites (Figs. 17, 18) contained one large crystalloid body, numerous amylopectin inclusions, and a typical apical complex consisting of a conoid, polar ring complex, rhoptries, micronemes and subpellicular microtubules.

DISCUSSION

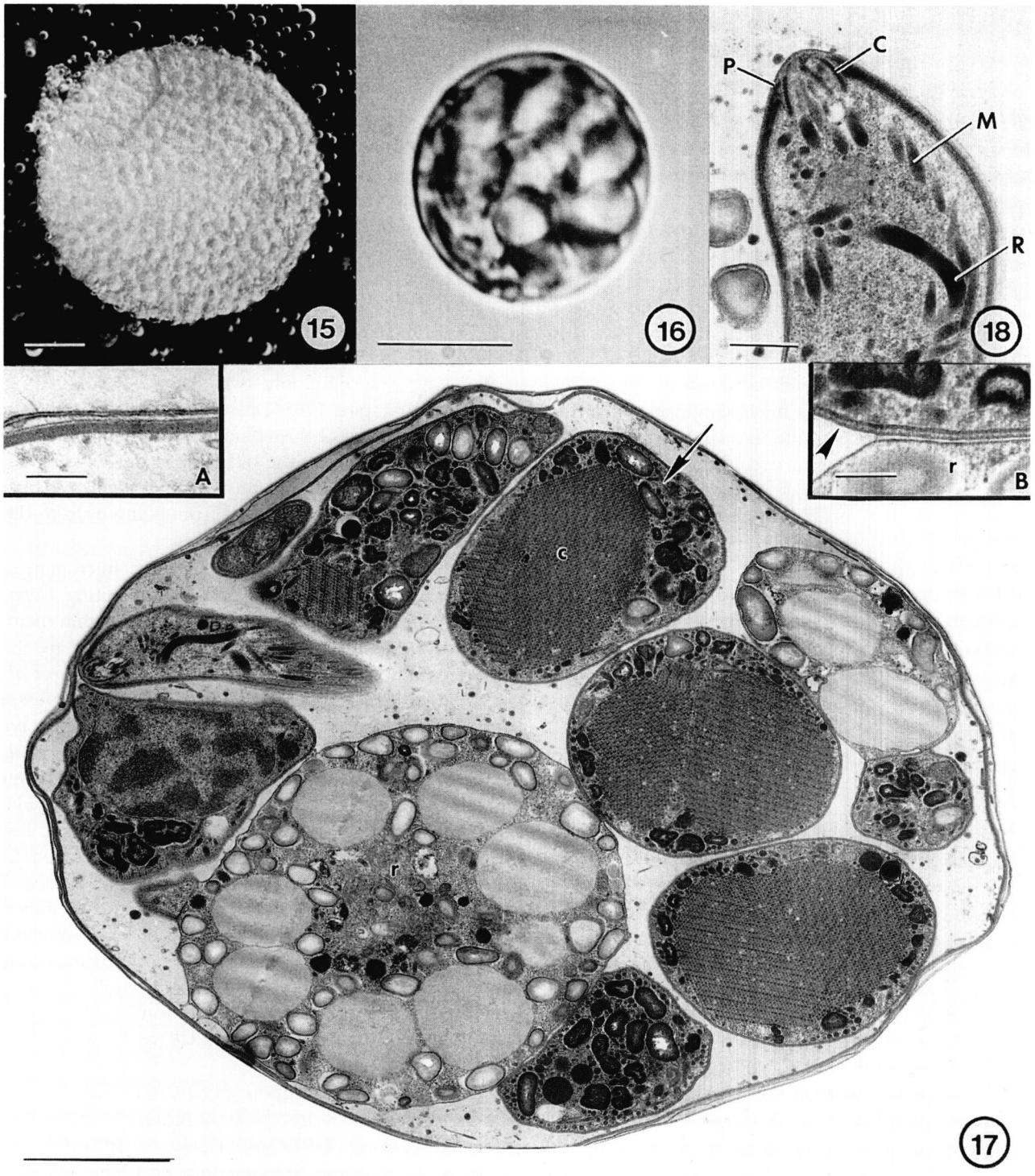
The observation that gamonts of *Hepatozoon sipedon* associate in close proximity to each other after emerging from erythrocytes within the blood meal of the mosquito is reported for a species of the genus for the first time. Lowichik *et al.* (1993) reported widely-dispersed gamonts of *H. mocassini* that travelled independently through the peritrophic membrane and penetrated the gut wall of the mosquito. Whether or not gamonts of *H. sipedon* maintain this close association as they penetrate the gut wall is not known. Syzygy, gametogenesis, the formation of a *Plasmodium*-like ookinete, and fertilization, which has been reported, perhaps erroneously (Siddall, 1995) for some Hepatozoon species to occur in the gut lumen of arthropods (Miller, 1908; Robin,



Figs. 10, 11. — Late sporogonic development of *H. sipedon* at 20 days PF. 10. Immature oocyst with small nuclei (n) near the periphery. Bar = 10 µm. 11. Higher magnification of a developing crystalloid body consisting of lipoprotein particles surrounded by rough endoplasmic reticulum. Bar = 0.2 µm.



Figs. 12-14. — Late sporogonic development of *H. sipedon* at 24 days PF. 12. Sporoblast, surrounded by a trilaminar membrane, containing large crystalloid bodies (*c*), amylopectin (arrow) and lipid (asterisks) inclusions, and a nucleus (*n*). Bar = 1 µm. 13. Crystalloid bodies are organized into paracrystalline arrays of small lipoprotien particles. Bar = 0.5 µm. 14. Developing sporoblasts with a trilaminar pellicle (arrow), large projections of cytoplasm, and micropores (arrowheads). Bar = 1 µm. Inset shows a micropore on the outer surface of the sporoblast membrane. Bar = 0.2 µm.



Figs. 15-18. — Mature oocysts and sporocysts of *H. sipedon* at 30 days PF. 15. Light micrograph of mature oocyst, containing over 500 sporocysts. Bar = 50 µm. 16. Phase contrast micrograph of a mature sporocyst with 8 sporozoites. Bar = 10 µm. 17. Cross-section through mature sporocyst, revealing sporozoites (arrows) that contain a single large crystalloid body (*c*), and a residual body (*r*) with amylopectin and lipid inclusions. Bar = 0.5 µm. Inset A shows the thick outer wall of the sporocyst composed of two layers. Bar = 0.2 µm. Inset B reveals that the residual body (*r*) is surrounded by a single membrane unlike the trilaminar pellicle (arrowhead) enclosing the mature sporozoite. Bar = 0.2 µm. 18. Mature sporozoite with an apical complex consisting of a conoid (C), polar ring complex (P), rhoptries (R) and micronemes (M). Bar = 0.2 µm.

1936; Lewis & Wagner, 1964), have not been observed in this or other ultrastructural studies of the gametogenesis of *Hepatozoon* species (Vivier *et al.*, 1972; Göbel & Krampitz, 1982; Bashtar *et al.*, 1984*b*; Lowichik *et al.*, 1993; Desser *et al.*, 1995).

The ultrastructural features of the gametogenic stages of *H. sipedon* in its definitive host, *Culex pipiens*, were generally similar to those of other species of *Hepatozoon*, including *H. domerguei* in *Anopheles stephensi* (see Vivier *et al.*, 1972), *H. aegypti* in *Culex pipiens molestus* (see Bashtar *et al.*, 1984*b*), and *H. mocassini* in *Aedes aegypti* (see Lowichik *et al.*, 1993), which utilize snakes as vertebrate hosts, *H. catesbianae* in *Culex territans*, which infects the erythrocytes of frogs (Desser *et al.*, 1995), and *H. erhardovae* in the flea *Xenopsylla cheopis*, which infects the leucocytes of voles (Göbel & Krampitz, 1982). In each of these species, syzygy involved the pairing of a microgamont and a macrogamont in the same parasitophorous vacuole and subsequent transformation of each gamont to a roughly spherical shape. Microgametogenesis resulted in the formation of microgametes that are characterized by a large electron-dense nucleus and, depending on the species, the presence of one or two flagella. The fate of those microgametes not involved in fertilization and the residual body of the microgamont is unknown, despite the fact that such residual structures are never seen after fertilization is complete, unlike that observed for *Haemogregarina balli*, in which such a residuum is present in the mature oocyst (Siddall & Desser, 1990).

Gametogenic and subsequent sporogonic development of *H. sipedon* occurred in the haemocoel of the definitive invertebrate host (see also Smith *et al.*, 1994), the most common site for sexual development of other members of this genus, including all of those infecting snakes (Landau *et al.*, 1970; Ball & Oda, 1971; Bashtar *et al.*, 1984*a*; Lowichik *et al.*, 1993), and many of those parasitizing lizards (Mackerras, 1962; Bashtar *et al.*, 1987) and mammals (Furman, 1966; Krampitz, 1981). Consistent with this localization of development is the formation of a non-motile zygote following syzygy and gametogenesis. Other members of the genus undergo sexual development and sporogony in the gut wall of a mite, as is the case with the lizard parasite, *H. lygosomarum* (see Allison & Desser, 1981) or a tick, as observed with the tortoise haemogregarine, *H. mauritanicum* (see Michel, 1973). The gut as a developmental location for gametogony and sporogony is similar to that of more derived adeleorins (Barta, 1989; Siddall & Desser, 1991; Siddall, 1995), including species of *Karyolysus* (see Svahn, 1975), *Desseria* (see Siddall & Desser, 1992), *Cyrilia* (see Lainson, 1981), *Haemogregarina* (see Siddall & Desser, 1990), and *Babesiosoma* (see Barta & Desser, 1989). In a phylogenetic analysis of the genus *Hepatozoon* and related haemogregarine taxa (Smith & Desser, in press), in which the developmental location of sporogony was used as a character, it was found that *H. lygosomarum*, *H. mauritanicum*, the remaining haemogregarine taxa and the dactylosomatids form a monophyletic group that is the sister clade to a monophyletic group of *Hepatozoon* species that develop in the haemocoel of the invertebrate host.

The number of microgametes formed from a microgamont also varies among species of *Hepatozoon* and has been used as a character in the phylogenetic analysis of the genus (Smith & Desser, in press). Four microgametes per microgamont have been commonly observed (Mackerras, 1962; Ball & Oda, 1971; Michel, 1973; Bashtar *et al.*, 1984*b*; Bashtar *et al.*, 1987; Lowichik *et al.*, 1993), whereas other studies, including this one, have revealed only two (Landau *et al.*, 1972; Göbel & Krampitz, 1982; Desser *et al.*, 1995). However, the inconsistency of this character among *Hepatozoon* species, coupled with the difficulty in determining the number of microgametes formed from each microgamont, precludes this character from being used to differentiate groups within the genus.

The number of flagella borne by each microgamete also provides phylogenetic data for separating *Hepatozoon* species, as again there is variation within members of the genus. Biflagellated microgametes are the most common state (Mackerras, 1962; Landau *et al.*, 1970; Ball & Oda, 1971; Lowichik *et al.*, 1993; Desser *et al.*, 1995), but uniflagellated microgametes have been reported (Göbel & Krampitz, 1982; Bashtar *et al.*, 1984*b*; Bashtar *et al.*, 1987), as have aflagellated forms (Michel, 1973). The biflagellated state is shared with the microgametes of eimeriorins, resulting in a less derived position for *Hepatozoon* in the phylogenetic analyses performed by Barta (1989), Siddall & Desser (1991) & Siddall (1995). The uniflagellated condition is similar to that observed in haemospororins (Garnham *et al.*, 1967; Desser, 1970) and lower adeleorins of the genus *Klossia* (see Moltmann, 1981), while the aflagellated forms are characteristic of more highly derived adeleorins (Lainson, 1981; Siddall & Desser, 1990, 1992; Barta, 1991).

The flagella of *Hepatozoon* species have been shown in the present study and those by Bashtar *et al.* (1984*b*), Lowichik *et al.* (1993) and Desser *et al.* (1995) to consist of unusual arrangements of single microtubules, including 9 + 1, 8 + 2, 8 + 1, 7 + 1, and 4 + 0 forms, all of which deviate from the classical 9 + 2 arrangement of doublet microtubules seen in the microgametes of other apicomplexans (Scholtyseck *et al.*, 1972). As suggested by Siddall & Desser (1990), such unusual arrangements of microtubules indicate that these structures are vestigial in nature, as such organelles would be unnecessary for adeleorin para-

sites that associate in close proximity during syzygy. In any case, the highly variable microtubule arrangements recorded for a single species of *Hepatozoon* likely precludes this feature for species description or differentiation.

Ultrastructural features of sporogonic stages of *H. sipedon* are consistent with those observed for *H. domerguei* (see Vivier *et al.*, 1972), *H. erhardovae* (see Göbel & Krampitz, 1982) *H. aegypti* (see Bashtar *et al.*, 1984*b*), *H. mocassini* (see Lowichik *et al.*, 1993) and *H. catesbianae* (see Desser *et al.*, 1995), despite the fact that for each species of parasite a different species of intermediate host is utilized. The early stages of nuclear division, observed as four nuclei contained within the immature oocyst (Fig. 9), have been illustrated previously by Vivier *et al.* (1972). They interpreted this stage as the final result of meiotic division of the zygote, a prelude to the mitotic division of nuclei that marks the first stage of sporoblast formation. Subsequently, several rounds of mitosis produce many sporocyst nuclei that are distributed near the periphery of the oocyst. Deep invaginations of the plasma membrane result in the formation of cytoplasmic protrusions, each containing one nucleus, that eventually bud off to form immature sporocysts (Bashtar *et al.*, 1984*b*).

In many studies of the sporogonic development of *Hepatozoon* species, small crystalloid bodies bound by rough endoplasmic reticulum appeared prior to the division of the immature oocyst into sporoblasts (20 days PF). It seems that these crystalloid bodies are the precursors of the larger forms seen in sporoblasts and sporocysts, as Bashtar *et al.* (1984*b*) observed deep invaginations of the oocyst membrane prior to sporoblast differentiation, with each resulting projection containing one nucleus and a number of small crystalloid bodies. Mature crystalloid bodies seen in mature sporozoites are composed of paracrystalline arrays of small lipoprotein-containing particles described originally from sporozoites of Leucocytozoon and *Haemoproteus* species (Desser & Trefiak, 1971; Trefiak & Desser, 1973). Small electron-dense inclusions that were abundant prior to the formation of crystalloid bodies decreased in number and eventually disappeared when crystalloid bodies were fully formed. These inclusions may serve as the lipoprotein precursor that is packaged into the particles that constitute each crystalloid body.

The presence of a trilaminar pellicle surrounding the developing sporocyst was reported in *H. sipedon* (Fig. 13) and *H. domerguei* (see Vivier *et al.*, 1972). The present study revealed that the mature sporozoites are invested by a trilaminar pellicle, whereas the residual body remaining after completion of sporocyst development was enclosed by a single plasma membrane. The formation of sporozoites from sporoblasts has been observed for other species of *Hepatozoon* (Bashtar *et al.*, 1984*b*; Lowichik *et al.*, 1993), and involves concurrent nuclear division of the sporoblast nucleus into presumptive sporozoite nuclei and the formation of sporozoite anlagen from cytoplasmic protrusions of the sporocyst.

Micropores on the surface of the sporoblast (Fig. 17) have been observed previously by Vivier *et al.* (1972). Their presence indicates that sporoblasts are still actively assimilating nutrients from the external environment, in this case the residuum contained within the walls of the oocyst, or perhaps material sequestered from the remains of the fat body of the mosquito host. In fact, mosquitoes containing 100 or more oocysts are usually devoid of eggs, indicating that resources normally allotted to egg production are probably utilized by the developing parasites.

Mature sporozoites of *H. sipedon* contain only one crystalloid body, similar to those of *H. catesbianae* (see Desser *et al.*, 1995). However, sporozoites of other *Hepatozoon* species (Bashtar *et al.*, 1984*b*; Lowichik *et al.*, 1993) and other adeleorins (Siddall & Desser, 1991; Siddall & Desser, 1993), contain two distinct crystalloid bodies. *Hepatozoon sipedon*, which requires both an anuran and ophidian host (Smith *et al.*, 1994) and *H. catesbianae*, which exclusively utilizes frogs as vertebrate hosts (Desser *et al.*, 1995), were hypothesized to be sister taxa in a phylogenetic reconstruction of *Hepatozoon* species (Smith & Desser, in press), and hence the reduction to one crystalloid body might represent an apomorphic feature of this clade. The ultrastructure of these bodies is similar to those reported from other *Hepatozoon* species and adeleorins.

Given the variability in the site of gametogenic and sporogonic development and the number and structure of the microgametes, as well as the conclusions made from phylogenetic hypotheses of the adeleorin genera, there is substantial information with which to justify splitting the genus *Hepatozoon*, an observation previously made by other workers (Ball & Oda, 1971; Barta, 1989) and reiterated by Smith & Desser (in press). There is little doubt that groups of *Hepatozoon* species displaying such variety in life history could be elevated to generic status, although the features that would constitute viable characters for separation would have to be determined. The lack of complete, reliable life cycle and ultrastructural data for the majority of *Hepatozoon* species, especially for those members which infect mammals, lizards, birds and crocodilians, currently precludes any division of this genus. Siddall & Desser (1992) stress the necessity for ultrastructural studies to fully document the life cycle of adeleorin parasites. Although ultrastructure may be useful in distinguishing among exis-

ting genera, the similarity of many of these stages among the *Hepatozoon* species investigated so far indicates that only certain features, including the number of microgametes produced per microgamont, the number of flagella borne by each microgamete, and the number and fine structure of crystalloid bodies, will be useful in further taxonomic studies.

ACKNOWLEDGEMENTS

We thank Henry Hong, Betty Kim and Chongxie Xiao for their comments on earlier drafts of the paper. Further recognition is due Henry Hong for his technical assistance and Andrea Lawson for her help in collecting mosquitoes at the Wildlife Research Station. This research was supported by The Natural Sciences and Engineering Research Council of Canada.

REFERENCES

ALLISON B. & DESSER S.S. Developmental stages of *Hepatozoon lygosomarum* (Doré, 1919) comb. n. (Protozoa: Haemogregarinidae), a parasite of a New Zealand skink, *Leiolopisma nigriplantare*. *Journal of Parasitology,* 1991, *67,* 852-858.

BALL G.H. Some blood sporozoans from east African reptiles. *Journal of Protozoology,* 1967, *14,* 198-210.

BALL G.H. & ODA S.N. Sexual stages in the life history of the hemogregarine *Hepatozoon rarefaciens* (Sambon & Seligman, 1907). *Journal of Protozoology,* 1971, *18,* 697-700.

BALL G.H., CHAO J. & TELFORD S.R. The life history of *Hepatozoon rarefaciens* (Sambon & Seligman, 1907) from *Drymarchon corais* (Colubridae), and its experimental transfer to *Constrictor constrictor* (Boidae). *Journal of Parasitology,* 1967, *53,* 897-909.

BARTA J.R. Phylogenetic analysis of the class Sporozoea (Phylum Apicomplexa Levine, 1970): Evidence for the independent evolution of heteroxenous life cycles. *Journal of Parasitology,* 1989, *75,* 195-206.

BARTA J.R. The Dactylosomatidae. *Advances in Parasitology,* 1991, *30,* 1-37.

BARTA J.R. & DESSER S.S. Development of *Babesiosoma stableri* (Dactylosomatidae, Adeleida; Apicomplexa) in its leech vector *(Batracobdella picta)* and the relationship of the dactylosomatids to the piroplasms of higher vertebrates. *Journal of Protozoology,* 1989, *36,* 241-253.

BASHTAR A.R., BOULOS R. & MEHLHORN H. *Hepatozoon aegypti* nov. sp.: 1. Life cycle. *Zeitschrift für Parasitenkunde,* 1984*a*, *70,* 29-41.

BASHTAR A.R., GHAFFAR, F.A. & MEHLHORN H. *Hepatozoon aegypti* nov. sp.: 3. Electron microscopic studies on the gamogony and sporogony inside the vector *Culex pipiens molestus*. *Zeitschrift für Parasitenkunde,* 1984*b*, *70,* 53-65.

BASHTAR A.R., ABDEL-GHAFFAR F.A. & SHAZLY M.A. Developmental stages of *Hepatozoon gracilis* (Wenyon, 1909) comb. nov., a parasite of the Egyptian skink, *Mabuya quinquetaeniata*. *Parasitology Research,* 1987, *73,* 507-514.

BOZZOLA J.J. & RUSSELL L.D. Electron microscopy: Principles and techniques for biologists. Jones and Bartlett Publishers, Boston, Massachusetts, USA, 1992.

DESSER S.S. The fine structure of *Leucocytozoon simondi*. I. Gametocytogenesis. *Canadian Journal of Zoology,* 1970, *48,* 331-336.

DESSER S.S. & TREFIAK W.D. Crystalline inclusions in *Leucocytozoon simondi*. *Canadian Journal of Zoology,* 1971, *49,* 134-135.

DESSER S.S., HONG H. & MARTIN D.S. The life history, ultrastructure, and experimental transmission of *Hepatozoon catesbianae* n. comb., an apicomplexan parasite of the bullfrog, *Rana catesbeiana* and the mosquito, *Culex territans* in Algonquin Park, Ontario. *Journal of Parasitology,* 1995, *81,* 212-222.

FURMAN D.P. *Hepatozoon balfouri* (Laveran, 1905): sporogonic cycle, pathogenesis, and transmission by mites to jerboa hosts. *Journal of Parasitology,* 1966, *52,* 373-382.

GARNHAM P.C.C., BIRD R.G. & BAKER J.R. Electron microscope studies of motile stages of malaria parasites. V. Exflagellation in *Plasmodium, Hepatocystis* and *Leucocytozoon*. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 1967, *61,* 58-68.

GÖBEL E. & KRAMPITZ H.E. Histologische Untersuchungen zur Gamogonie und Sporogonie von *Hepatozoon erhardovae* in experimentell infizierten Rattenflöhen *(Xenopsylla cheopis)*. *Zeitschrift für Parasitenkunde,* 1982, *67,* 261-271.

KRAMPITZ H.E. Development of *Hepatozoon erhardovae* Krampitz, 1964 (Protozoa: Haemogregarinidae) in experimental mammalian and arthropod hosts. II. Sexual development in fleas and sporozoite indices in xenodiagnosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 1981, *75,* 155-157.

LAINSON R. On *Cyrilia gomesi* (Neiva and Pinto, 1926) gen. nov. (Haemogregarinidae) and *Trypanosoma bourouli* Neiva and Pinto, in the fish *Synbranchus marmoratus:* simultaneous transmission by the leech *Haementeria lutzi*. *In:* Parasitological Topics, Special Publication No. 1, Society of Protozoologists. Canning E.U. (ed.), Lawrence, Kansas, USA, 1981, 150-158.

LANDAU I., CHABAUD A.G., MICHEL J.C. & BRYGOO E.R. Mise en évidence d'un double mode de transmission chez un *Hepatozoon* de Reptiles malgaches. *Comptes Rendus de l'Académie des Sciences,* 1970, *270,* 2308-2310.

LANDAU I., MICHEL J.C., CHABAUD A.G. & BRYGOO E.R. Cycle biologique d'*Hepatozoon domerguei*; discussion sur les caractères fondamentaux d'un cycle de Coccidie. *Zeitschrift für Parasitenkunde,* 1972, *38,* 250-270.

LEVINE N.D. The protozoan phylum Apicomplexa. CRC Press Incorporated, Boca Raton, Florida, 1988.

LEWIS J.E. & WAGNER E.D. *Hepatozoon sauromali* sp. n., a haemogregarine from the Chuckwalla (*Sauromalus* spp.) with notes on the life history. *Journal of Parasitology,* 1964, *50,* 11-14.

LOWICHIK A., LANNERS N.L., LOWRIE R.C. & MEINERS N.E. Gametogenesis and sporogony of *Hepatozoon mocassini* (Apicomplexa: Adeleina: Hepatozoidae) in an experimental mosquito host, *Aedes aegypti*. *Journal of Eukaryotic Microbiology,* 1993, *40,* 287-297.

MACKERRAS M.J. The life history of a *Hepatozoon* (Sporozoa: Adeleidea) of varanid lizards in Australia. *Australian Journal of Zoology,* 1962, *10,* 35-44.

MICHEL J.C. *Hepatozoon mauritanicum* (Et. et Ed. Sergent, 1904) n. comb., parasite de *Testudo graeca*: redescription de la sporogonie chez *Hyalomma aegyptium* et la schizogonie tissulaire d'après le matérial d'E. Brumpt. *Annales de Parasitologie Humaine et Comparée,* 1973, *48,* 11-21.

MILLER W.W. *Hepatozoon perniciosum* n. g., n. sp., a haemogregarine pathogenic for white rats; with a brief description of the sexual cycle in the intermediate host, a mite (*Laelaps echidninus* Berlese). *Bulletin of the Hygiene Laboratory of Washington,* 1908, *46,* 51-123.

MOHAMMED A.H.H. & MANSOUR N.S. The haemogregarine complex (an analytical systematic review). *Bulletin of the Faculty of Science, Cairo University,* 1959, *35,* 39-51.

MOLTMANN U.G. Light and electron microscopic studies on *Klossia helicina* (Coccidia): development of gamonts in snail kidney tissue cultures and sporogony in the natural host. *Protistologica,* 1981, *17,* 185-197.

REYNOLDS E.S. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *Journal of Cell Biology,* 1963, *17,* 208.

ROBIN L.A. Cycle évolutif d'un *Hepatozoon* de *Gecko verticillatus*. *Annales de l'Institut Pasteur,* 1936, *56,* 376-394.

SCHOLTYSECK E., MEHLHORN H. & HAMMOND D.M. Electron microscope studies of microgametogenesis in Coccidia and related groups. *Zeitschrift für Parasitenkunde,* 1972, *38,* 95-131.

SIDDALL M.E. Phylogeny of adeleid blood parasites with a partial systematic revision of the haemogregarine complex. *Journal of Eukaryotic Microbiology,* 1995, *42,* 116-125.

SIDDALL M.E. & DESSER S.S. Gametogenesis and sporogonic development of *Haemogregarina balli* (Apicomplexa, Adeleina: Haemogregarinidae) in the leech *Placobdella ornata*. *Journal of Protozoology,* 1990, *37,* 511-520.

SIDDALL M.E. & DESSER S.S. Merogonic development of *Haemogregarina balli* (Apicomplexa, Adeleina: Haemogregarinidae) in the leech *Placobdella ornata* (Glossiphoniidae), its transmission to a chelonian intermediate host and phylogenetic implications. *Journal of Parasitology,* 1991, *77,* 426-436.

SIDDALL M.E. & DESSER S.S. Ultrastructure of gametogenesis and sporogony of *Haemogregarina* (sensu lato) *myoxocephali* (Apicomplexa: Adeleina) in the marine leech *Malmiana scorpii*. *Journal of Protozoology,* 1992, *39,* 545-554.

SIDDALL M.E. & DESSER S.S. Ultrastructure of merogonic development of *Haemogregarina* (sensu lato) *myoxocephali* (Apicomplexa: Adeleina) in the marine leech *Malmiana scorpii* and localization of infective stages in the salivary cells. *European Journal of Protistology,* 1993, *29,* 191-201.

SMITH T.G. The genus *Hepatozoon* (Apicomplexa: Adeleina). *Journal of Parasitology,* 1996, *82,* 565-585.

SMITH T.G. & DESSER S.S. Phylogenetic analysis of the genus *Hepatozoon* (Apicomplexa: Adeleorina). *Systematic Parasitology,* in press.

SMITH T.G., DESSER S.S. & MARTIN D.S. The development of *Hepatozoon sipedon* n. sp. (Apicomplexa: Adeleina: Hepatozoidae) in its natural host, the Northern water snake *(Nerodia sipedon sipedon),* the culicine vectors, *Culex pipiens* and *Culex territans,* and an intermediate host, the Northern leopard frog *(Rana pipiens). Parasitology Research,* 1994, *80,* 559-568.

SPURR A.R. A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructural Research,* 1969, *26,* 31.

SVAHN K. Blood parasites of the genus *Karyolysus* (Coccidia, Adeleina) in Scandinavian lizards. Description and life cycle. *Norwegian Journal of Zoology,* 1975, *23,* 277-295.

TELFORD S.R. Haemoparasites of reptiles. *In:* Diseases of Amphibians and Reptiles. Hoff G.L., Frye F.L. & Jacobson E.R. (eds), Plenum Press, New York, USA, 1984, 385-517.

TREFIAK W.D. & DESSER S.S. Crystalloid inclusions in species of *Leucocytozoon, Parahaemoproteus,* and *Plasmodium*. *Journal of Protozoology,* 1973, *20,* 73-80.

VIVIER E., PETITPREZ A. & LANDAU I. Observations ultrastructurales sur la sporoblastogenèse de l'hémogrégarine, *Hepatozoon domerguei,* Coccidie, Adeleidea. *Protistologica,* 1972, *8,* 315-333.

Reçu le 1er février 1997
Accepté le 14 mars 1997