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Life cycle of *Hepatozoon mehlhorni* sp. nov. in the viper *Echis carinatus* and the mosquito *Culex pipiens*

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Abstract. *Hepatozoon mehlhori* sp. nov. and its developmental stages from the tissues of the Egyptian viper *Echis carinatus* and the mosquito *Culex pipiens* are described. The erythrocytic parasites were differentiated into the small form (trophozoite) measuring $14.5 \pm 0.6 \times 4 \pm 0.12$ µm and the mature form (gametocyte) measuring $17.2 \pm 1.6 \times 5.4 \pm 0.5$ µm. Merogony took place in the pulmonary endothelial cells and in the parenchyma cells of the liver and spleen of the infected vipers. Two types of meront were found. The large meronts (macromeronts) were $30.2 \pm 1.73 \times 22.6 \pm 1.2$ µm in size and yielded 16–40 (average, 28) micromerozoites measuring $17.2 \pm 0.7 \times 5 \pm 0.15$ µm. The small meronts (micromeronts) measured $18.2 \pm 0.6 \times 13.5 \pm 0.5$ µm and yielded 2–14 (average, 8) macromerozoites that were $15.1 \pm 0.12 \times 6.2 \pm 0.8$ µm in size. After syzygy in the haemocoel of the mosquito, the microgamont produced four uniflagellate microgametes ($6.4 \pm 0.3 \times 4.5 \pm 0.5$ µm in size, with a short flagellum measuring 3.2 ± 0.1 µm); on the 3rd day post-infection (p.i.)., one of these fertilized the macrogamete, giving rise to the zygote. The oocyst developed from the zygote on the 5th day p.i. and measured $135 \pm 2.6 \times 120 \pm 1.8$ µm. About 11–60 (average, 35) sporoblasts were formed by centripetal invaginations from each oocyst on the 8th day p.i. and developed into sporocysts on the 14th day p.i. Inside each sporocyst, 5–12 (average, 8) sporozoites, each measuring $12.6 \pm 1.2 \times 4.1 \pm 0.3$ µm, developed on the 16th day p.i. According to the above-mentioned characteristics the parasite was recorded as being a new species and was named *Hepatozoon mehlhorni*. Experimental transmission was accomplished by i.p. inoculation of the infectious stages (sporozoites) into uninfected vipers and led to the appearance of blood stages at 4–6 weeks p.i.

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Abbreviations: *BLC*, Blood capillary; *DMS*, developing merozoites; *DSP*, developing sporoblasts; *E*, erythrocyte; *F*, flagellum; *HC*, host cell; *HN*, host nucleus; *M*, Meront; *MA*, macrogamont/macrogamete; *MG*, Microgamete; *MIG*, microgamont; *MS*, merozoites; *N*, nucleus; *NG*, nucleus of the microgamete; *OC*, oocyst; *P*, erythrocytic parasite; *PV*, Parasitophorous vacuole; *SP*, sporoblast (s); *SPC*, sporocyst (s); *SPR*, sporozoite (s); *ZY* zygote (young oocyst)

Haemogregarines are cosmopolitan apicomplexan blood parasites belonging to the eucoccidian suborder Conoidina (Mehlhorn 1988). They have an obligatory heteroxenous life cycle in which merogony takes place in the vertebrate host and gamogony and sporogony occur in the haematophagous invertebrate host.

Haemogregarines infecting Egyptian reptiles are known through numerous taxonomic and histological studies (Bashtar et al. 1984, 1987; Abdel-Ghaffar 1985; Bashtar and Abdel-Ghaffar 1987). Although the saurian haemogregarines have been extensively studied (Robin 1936; Mackerras 1962; Landau et al. 1972; Allison and Desser 1981; Bashtar et al. 1987; Elwasila 1989; Shanavas and Ramachandran 1990), few life histories of snake haemogregarines have been described (Ball et al. 1967, 1969; Bashtar et al. 1984; Nadler and Miller 1984; Sinha 1986).

The present report describes the complete life cycle of a new haemogregarine species parasitizing the Egyptian viper *Echis carinatus* and the mosquito *Culex pipiens*.

Materials and methods

Animals

Snakes. A total of 75 adult male and female *Echis carinatus* (Viperidae, Squamata; Anderson 1898) were collected from the Siwah and Bahariah oases in Egypt. To protect the vipers against possible insect vectors of the parasites, they were maintained in glass cages with sand and wire-gaze tops in an animal room at a temperature range of 27°–32° C. Water and food (small laboratory-reared *Mus musculus*) were provided regularly.

Vectors. Mosquitoes of the species *Culex pipiens* (Culicidae, Diptera) were used as the invertebrate vector. They were successfully

colonized at room temperature in the laboratory. Mosquito larvae were fed with Tetramin (commercial fish food), and the adults were given 5%–10% sugar solution before being allowed to feed on infected snakes.

Parasites. Vipers were tested for haemogregarine infection by the preparation of a thin blood film from blood obtained from each viper by clipping off its tail tip. Blood films were air-dried, fixed in absolute methanol (5 min) and stained with 3% Giemsa solution in phosphate buffer (pH 7.3) for at least 30 min. Infected vipers were isolated and divided into two groups; snakes in the first group were exposed to the mosquitoes, and those in the second were decapitated and dissected for the detection of merogonic developmental stages within their internal organs.

Fixation, embedding and microscopic examination. Excised from the highly infected vipers, pieces measuring about 3 mm were taken from the liver, lung, spleen, brain, kidney, heart, stomach, ileum and skeletal muscles, as well as from the mosquito abdomen, and were immediately fixed in 3% (v/v) glutaraldehyde buffered in 0.1 M sodium cacodylate (pH 7.3) for at least 4 h at 4° C. After 4–5 washings in the cacodylate buffer for 10–15 min each, the specimens were dehydrated in a series of alcohols, cleared in butanol, embedded in Paraplast and sectioned on a rotary microtome. The sections were stained with hematoxylin and eosin. The stained sections and blood smears were examined with an Olympus research photo-microscope. The results were recorded as mean values $\pm$ SD, with the number of measurements being given in parentheses ($n = 50$).

Results

Natural incidence and levels of parasitaemia

Of the 75 *Echis carinatus* collected, 48 (64%) were found to be infected with haemogregarines on initial examination. The parasitaemia in some vipers was very high (800–1,000 erythrocytes), on one occasion reaching a level of 96% (Figs. 1, 2).

Blood stages of the parasite

The parasites in the peripheral blood were usually intracellular (Figs. 1, 2), but free extracellular parasites were occasionally seen in some smears. The intracellular parasites parasitized only the red blood cells of the host. Occasionally, double and triple infections were observed, especially in cases of high parasitaemia (Figs. 1, 2). None of the leucocytes were parasitized. Most parasites were enclosed within a clear parasitophorous vacuole, but sometimes no space or membrane was observed between the parasite and the host cell cytoplasm.

The blood stages of the parasite were differentiated into two forms. The first, a small form, measured 14.5 ± 0.6 µm in length and 4 ± 0.12 µm in width; it was usually smaller than the normal host cell and represented a merozoite (trophozoite) that had just completed the process of penetration. The second, a mature form, was sausage-shaped and slightly bowed; it measured 17.2 ± 1.6 µm in length and 5.4 ± 0.5 µm in width (Figs. 1, 2) and represented a mature gametocyte. This large stage had slightly rounded ends that usually bent inwards around the host cell nucleus, which was laterally displaced to the opposite side of the parasite. Uninfected red blood cells measured $13.6 \pm 0.9 \times 7.9 \pm 0.8$ µm; infected erythrocytes showed hypertrophy and faintly stained cytoplasm (Figs. 1, 2) and measured $21 \pm 0.6 \times 8.1 \pm 1.2$ µm.

The parasite's cytoplasm usually stained faint pink to slightly red and contained some electron-dense granules and a few vacuoles. The nucleus of the parasite stained red with Giemsa stain and often appeared as a roughly transverse band containing some electron-dense granules and measuring $6.3 \pm 0.6 \times 5.4 \pm 0.5$ µm (Figs. 1, 2).

Merogony and merozoites

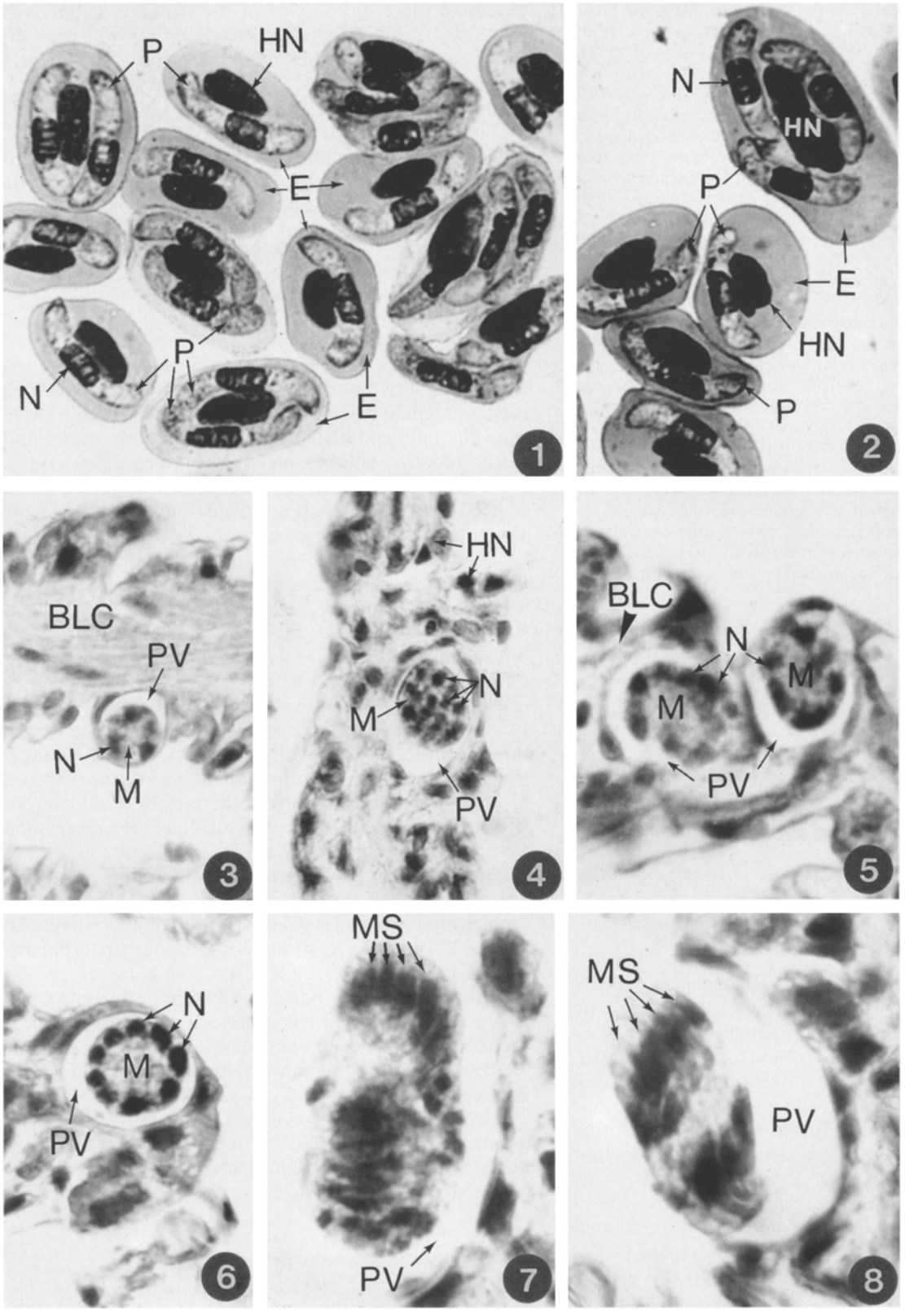
Meronts in different developmental stages were observed in the parenchyma cells of the liver and spleen as well as in the lung endothelial cells lining the air spaces and in the blood capillaries of infected vipers (Figs. 3–17). Merogonic stages were not seen in the circulating blood cells, skeletal muscles, brain, heart or kidney or in the alimentary canal of infected vipers.

The smallest meront seen within the parenchyma and endothelial cells of infected vipers (Fig. 3) measured $9 \pm 0.7 \pm 7.5 \pm 0.4$ µm, whereas the largest (Figs. 12, 14) measured $27.6 \pm 0.7 \times 17.5 \pm 0.5$ µm. The nucleus of the meront usually divided many times, producing many new nuclei, which migrated to the periphery of the meront (Figs. 5, 6, 9, 10). The first sign of merozoite formation was a thickening and elevation of the outer border of the meront in an area overlying a nucleus, accompanied by the commencing production of narrow merozoites as finger-like outgrowths from the surface of the meront (Figs. 7, 8, 12, 15, 16), a process that has been designated ectomerogony (Hammond 1973).

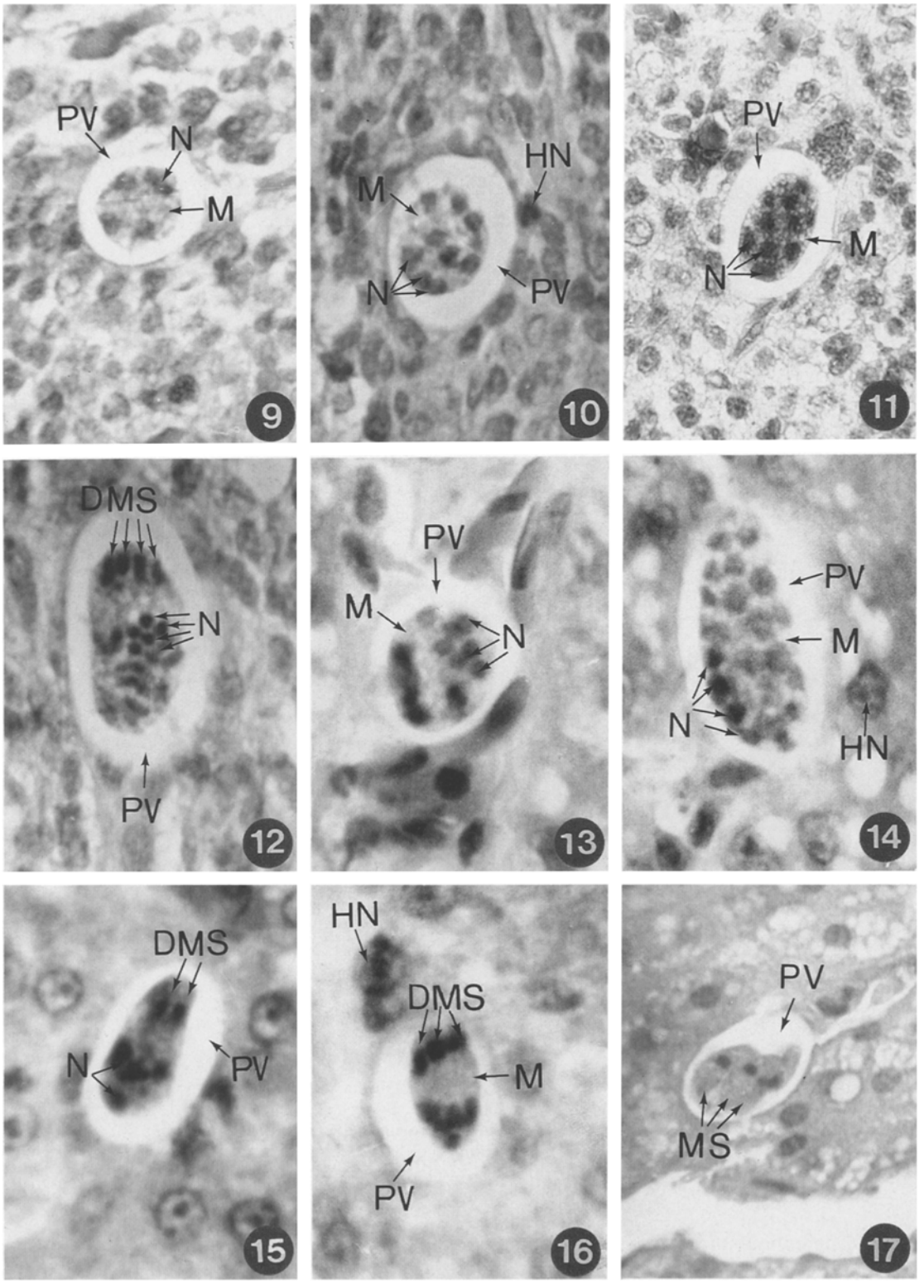
As observed in the present investigation, merogony resulted in two forms at meront. The small meronts (micromeronts) were $18.2 \pm 0.6 \times 13.5 \pm 0.5$ µm in size and yielded 2–14 (average, 8) large merozoites (macromerozoites) measuring $15.1 \pm 0.12 \times 6.2 \pm 0.8$ µm (Figs. 15–17). Such merozoites had a pink-staining hyaline cytoplasm and a well-marked transverse nucleus that measured $6.5 \pm 0.3 \times 6.2 \pm 0.8$ µm. The large meronts (macromeronts) reached a maximal diameter of 30.2 ± 1.72 µm and a length of 22.6 ± 1.2 µm and yielded 16–40 (average, 28) elongated merozoites (micromerozoites). Each merozoite was $17.2 \pm 0.7 \times 5 \pm 0.15$ µm in size and contained an elongated nucleus measuring $6.3 \pm 0.4 \times 5 \pm 0.1$ µm (Figs. 7, 8).

Gamogony

Some merozoites differentiated into gametocytes (vermicules) in the circulating erythrocytes of the vipers. During the 1st day after the mosquitoes had fed on infected blood, the parasites were released from their erythrocytes, penetrated the midgut of the mosquito and appeared in the haemocoel (Fig. 18). On the 2nd day post-infection (p.i.), the parasites were seen to associate in pairs, lying side by side in syzygy within a parasitophor-

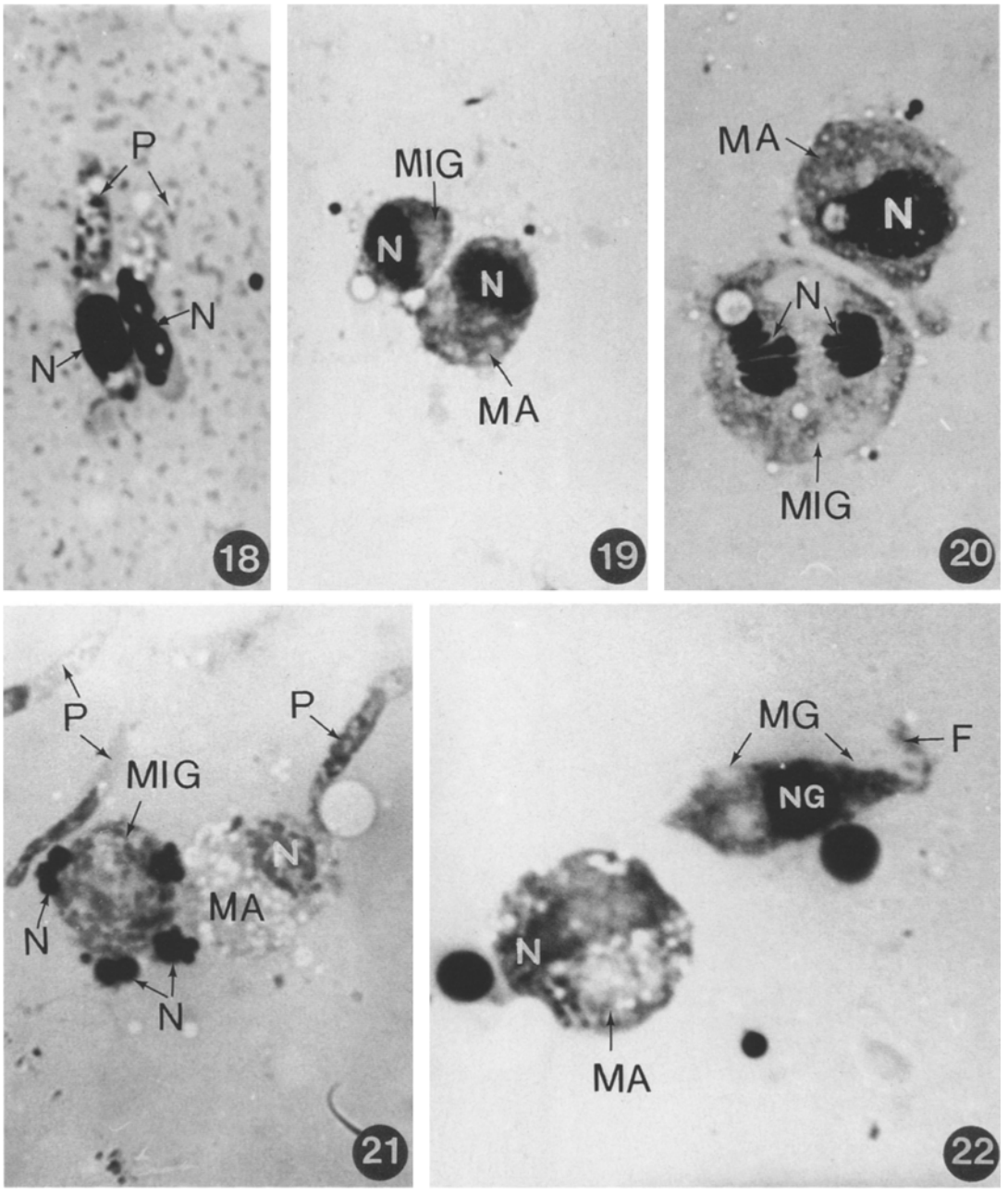


Figs. 1, 2. Giemsa-stained blood smears of the viper showing intraerythrocytic parasites (*P*). Double and triple infections are seen. ×2,500. **Figs. 3–8.** Merogonic stages in the lung endothelial cells of the viper. **Fig. 3.** Developing meront (*M*) with 3 nuclei (*N*). ×1,800. **Fig. 4.** A macromeront (*M*) with irregularly situated numerous nuclei (*N*). ×1,500. **Figs. 5, 6.** Micromeronts (*M*) with peripherally arranged daughter nuclei (*N*). ×2,200. **Figs. 7, 8.** Longitudinal section of macromeronts (*M*), showing the formation of merozoites (*MS*). ×2,200



Figs. 9–12. Merogonic stages in the spleen parenchyma cells of the viper. **Fig. 9.** Micromeront (*M*) with four nuclei (*N*) and a large parasitophorous vacuole (*PV*). ×1,800. **Figs. 10, 11.** Macromeronts (*M*) with irregularly situated daughter nuclei (*N*). ×1,800. **Figs. 12.** Commencing production of developing merozoites (*DMS*) as finger-like outgrowths from the ends of the macromeront (*M*). ×2,000

Figs. 13–17. Merogonic stages in the liver parenchyma cells of the viper. **Fig. 13.** A micromeront (*M*) with randomly distributed nuclei (*N*). ×2,000. **Fig. 14.** A macromeront (*M*) with numerous irregularly situated nuclei (*N*). ×2,000. **Figs. 15, 16.** Micromeronts (*M*) showing the budding of developing merozoites (*DMS*) from their ends. ×2,000. **Fig. 17.** Longitudinal section of free macromerozoites (*MS*) within a parasitophorous vacuole (*PV*). ×2,000



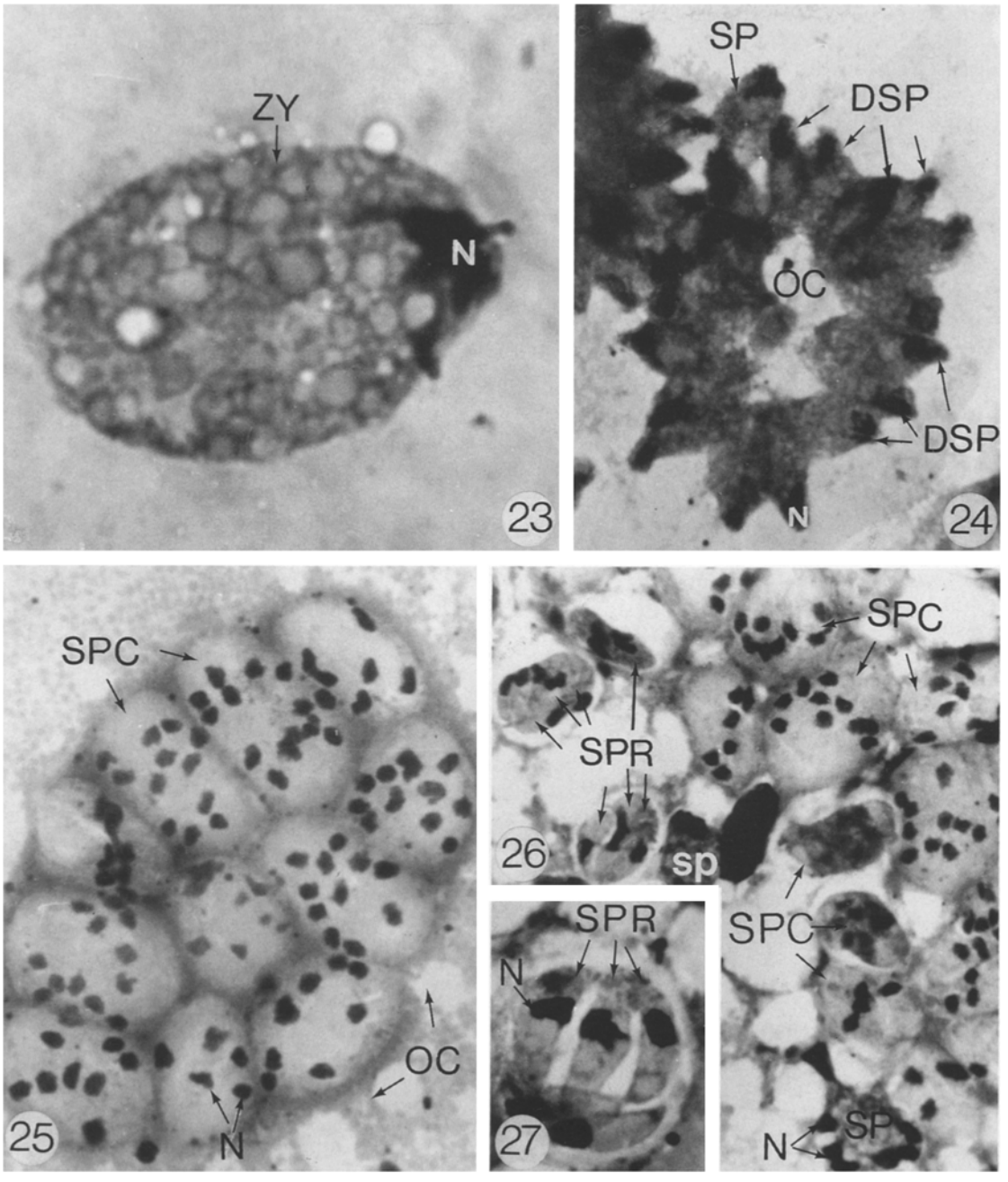
Figs. 18–22. Gamogonic stages of *Hepatozoon mehlhorni* in the mosquito myxocoel (Giemsa stain). **Fig. 18.** Two non-differentiated vermicules (*P*) on the 1st day p.i. ×2,200. **Fig. 19.** A microgamont (*MIG*) in the vicinity of a macrogamont (*MA*) at the beginning of syzygy. ×2,200. **Fig. 20.** The division of the microgamont nucleus (*MIG*) into 2 nuclei (*N*) during syzygy on the 2nd day p.i. ×2,200. **Fig. 21.** Syzygy of a microgamont (*MIG*) with 4 nuclei (*N*) and a macrogamont (*MA*) on the 3rd day p.i. ×2,000. **Fig. 22.** Free mono-flagellate microgamete (*MG*) with a short flagellum (*F*) begins to fertilize the macrogamete (*MA*). ×2,200

ous vacuole, and began to differentiate into micro- and macrogamonts (Fig. 19).

On the 3rd day p.i., the paired gamonts were clearly differentiated into a small oval or spherical microgamont and a large spherical macrogamont. The microgamont measured $13 \pm 0.13 \times 11.5 \pm 0.6$ µm and the macrogamont reached a size of $16.3 \pm 0.8 \times 13.2 \pm 0.25$ µm. In the meantime, the nucleus of the microgamont had divided to yield 2–4 daughter nuclei (Figs. 20, 21), resulting in the formation of 2–4 monoflagellate microgametes. The microgamete was pear-shaped and had a pointed tip and measured $6.4 \pm 0.3 \times 4.5 \pm 0.5$ µm. It had a central nucleus and a short flagellum reaching 3.2 ± 0.1 µm in length (Fig. 22). At the same time, the macrogamont increased markedly in size and attained a rounded to oval shape, giving rise to a single mature macrogamete (Fig. 22) measuring $22.8 \pm 1.6 \times 20.7 \pm 1.2$ µm.

Sporogony

On the 4th day p.i., evidence of fertilization was observed when one microgamete was found in the vicinity



Figs. 23–27. Sporogonic stages of *Hepatozoon mehlhorni* in the mosquito myxocoel. **Fig. 23.** A zygote (*ZY*) or young oocyst with an eccentric nucleus (*N*) on the 4th day p.i. ×2,500. **Fig. 24.** An oocyst (*OC*) showing the formation of centripetal invaginations, which then form the developing sporoblasts (*DSP*) on days 5–8 p.i. ×2,000. **Fig. 25.** An oocyst (*OC*) with 11 sporocysts (SPC) containing irregularly situated daughter nuclei (*N*) on the 12th day p.i. ×2,000. **Fig. 26.** Scattered sporocysts (*SPC*), some with free sporozoites (*SPR*). ×1,800. **Fig. 27.** Mature sporocysts (*SPC*) with free sporozoites (*SPR*) on the 16th day p.i. ×2,200

of a macrogamete (Fig. 22); the microgamete apparently then fertilized the macrogamete, giving rise to the zygote (Fig. 23), which had an oval shape and measured about $36.2 \pm 1.3 \times 23.5 \pm 0.8$ μm.

Beginning at days 5–8 p.i., the zygote developed into an oocyst, since it grew considerably in size ($135 \pm 2.6 \times 120 \pm 1.8$ μm) due to rapid accumulation of large quantities of reserve food materials. During growth of the oocyst, its large prominent nucleus started division, leading to the formation of numerous nuclei. Beginning at days 8–10 p.i., the oocyst started forming numerous centripetal invaginations at its outer boundary. These invaginations formed deeper, finger-like infoldings and each lobe finally incorporated a nucleus, thus giving rise to a developing sporoblast (Fig. 24). These sporoblasts separated from the oocyst, leaving a residual body.

At days 10–12 p.i., the sporoblast nucleus underwent several divisions and the newly formed daughter nuclei were arranged indiscriminately in the sporoblast (Figs. 25, 26). At the completion of sporulation, the

sporoblasts had developed into sporocysts (Figs. 26, 27); about 11–60 (average, 35) sporocysts were observed per oocyst. Mature sporocysts measured $16.8 \pm 1.3 \times 13.1 \pm 0.6$ µm and usually contained 5–12 (average, 8) sporozoites (Figs. 26, 27). On days 14–16 p.i., most sporocysts displayed fully formed sporozoites. Each sporozoite had an elongated, curved body with pointed ends that measured $12.6 \pm 1.2 \times 4.1 \pm 0.3$ µm (Figs. 26, 27).

Experimental transmission of infection

Experimental transmission of the infectious stage (sporozoites) from mosquitoes to uninfected vipers was accomplished by intraperitoneal inoculation of a homogenate of whole infected mosquitoes at 20–25 days after the insects had fed on infected blood. At 4–6 weeks p.i., parasites identical to blood stages previously described in natural infection appeared in the erythrocytes of vipers.

Discussion

The parasite described in the present report is the first adeleid protozoan parasite to be recorded in the Egyptian viper *Echis carinatus*, which is widely distributed in Egypt, Algeria, Sudan, Syria and Eastern Arabia (Anderson 1898). Experimental transmission of the infectious stage (sporozoite) from the invertebrate host *Culex pipiens* to uninfected vipers was achieved through intraperitoneal inoculation of sporozoites in physiological saline solution. Furthermore, as the present parasite showed all of the diagnostic characteristics of the genus *Hepatozoon*, we report it as being a new species and assign to it the name *H. mehlhorni* sp. nov.

H. mehlhorni infects only the erythrocytes of the vertebrate host; this is the common position for haemogregarines in all reptiles. Haemogregarines have also occasionally been seen in leucocytes of mammals (Killick-Kendrick 1984; Conceiçao-Silva et al. 1988), birds (Fantham et al. 1942) and fishes (Kirmse 1979; Barber et al. 1987). The infected erythrocytes were hypertrophied and showed considerable distortion. Hypertrophy has been reported in erythrocytes infected with *Hepatozoon* spp. (Mackerras 1962; Ball et al. 1967, 1969; Bashtar et al. 1987). Also, Beyer (1977, 1982) has noted hypertrophy of erythrocytes infected with *Karyolysus*.

In the present study, *H. mehlhorni* occurred as merogonic stages in the parenchyma cells of the liver and spleen as well as in the pulmonary endothelial cells of infected vipers. Similar observations have previously been reported for *H. mesnili* (Robin 1936), *H. mauritanicum* (Michel 1973) and *Haemogregarina najae* (Bashtar and Abdel-Ghaffar 1987). In contrast, Beyer et al. (1983) have observed a large haemogregarine meront in the testis of the lizard *Lacerta saxicola*. However, the liver has been reported to be the most common position of merogonic developmont for the genus *Hepatozoon* (Mackerras 1962; Lewis and Wagner 1964; Allison and Desser 1981; Nadler and Miller 1984; Bashtar et al. 1987; Shanavas and Ramachandran 1990). Nonetheless, the lung has also been noted as being the site of merogony for many other *Hepatozoon* species (Krampitz 1964; Ball et al. 1967, 1969; Davidson and Calpin 1976; Bashtar et al. 1984).

The presence of two distinct types of meront, which were referred to as micro- and macromeronts on the basis of their morphology rather than their fine structure, in the present parasite (*H. mehlhorni*) was clearly in line with previous studies on other species of the genus *Hepatozoon* (Mackerras 1962; Ball et al. 1967, 1969; Michel 1973; Bashtar et al. 1984, 1987; Conceiçao-Silva et al. 1988; Shanavas and Ramachandran 1990). Moreover, Hawking et al. (1948) have claimed that the presence of two types of meront is a fundamental feature of the order Coccidia; these authors even thought that in mammalian *Plasmodia* infections, in which only one of the two types of meront is known, the other might also be present, albeit not identified, or be reduced to hidden forms. At the same time, the respective roles of the two types of meronts and their merozoites in the life cycle of haemogregarines were discussed. Most investigators have postulated that one type of merozoite undergoes the asexual cycle and gives rise to trophozoites and, eventually, to meronts and to merozoites, whereas the other type initiates the sexual cycle and yields gametocytes (Mackerras 1962; Furman 1966). However, there has been no experimental evidence that meronts and gametocytes result from different types of merozoites (Ball et al. 1967).

Information on the vector-phase development of *Hepatozoon* spp. is rather scarce. This has made our understanding of sporogony in these parasites even poorer than that of merogony. In general, the invertebrate vectors of haemogregarines have presented the most difficult problem hindering the progress of research on this groups (Levine 1982). Nevertheless, a large number of invertebrate vectors are known to transmit *Hepatozoon* parasites to their vertebrate hosts, such as fleas (Krampitz 1964; Göbel and Krampitz 1982), mites (Furman 1966; Allison and Desser 1981; Shanavas and Ramachandran 1990), ticks (Michel 1973), mosquitoes (Robin 1936; Mackerras 1962; Ball et al. 1967, 1969; Nadler and Miller 1984; Bashtar et al. 1984, 1987), sandflies (Ayala 1970) and tsetes flies (Hoare 1932). On the other hand, leeches have been suggested to be the vectors of the genus *Haemogregarina* in turtles (Paterson and Desser 1976) and in crocodiles (Khan et al. 1980).

The development of gamogonic stages of *H. mehlhorni* in the haemocoel of the vector in the present study was similar to findings in other *Hepatozoon* species (Mackerras 1962; Ball et al. 1967, 1969; Bashtar et al. 1984, 1987). In contrast, *H. mesnili* (Robin 1936), *H. mauritanicum* (Michel 1973) and *H. lygosomarum* (Allison and Desser 1981) have been shown to undergo syzygy and gamogony in the stomach of their vectors.

Controversy exists on the process of gametogenesis among haemogregarines in terms of the number of microgametes produced by each microgamont. Members of the suborder Adeleidea are characterized by the production of only a few (maximally four) microgametes. The formation of four microgametes from a single microgamont in *H. mehlhorni* is in accordance with other

observations in the genus *Hepatozoon* (Mackerras 1962; Michel 1973; Bashtar et al. 1984, 1987). However, this finding contradicts the results obtained by Robin (1936), Furman (1966) and Göbel and Krampitz (1982) in other *Hepatozoon* species; these authors suggested that the entire nucleus of the microgamont is incorporated into one microgamete without cytoplasmic divisions.

The present study revealed the presence of only mono-flagellate haemogregarine microgametes. This has also been reported for other *Hepatozoon* species (Göbel and Krampitz 1982; Bashtar et al. 1984, 1987). In contrast, the microgametes of *H. breinli* (Mackerras 1962) and *H. rarefaciens* (Ball and Oda 1971) have two flagella, and Michel (1973) has, found non-flagellate microgametes in *H. mauritanicum*.

Sporogony was elucidated for only a few of the known species of haemogregarines. Chatton and Roubaud (1913) first called attention to the characteristic type of *Hepatozoon* sporogony, in which large oocysts containing many sporocysts that produce numerous sporozoites were always seen. In *H. mehlhorni*, sporoblast formation in the oocyst begins with the development of centripetal invaginations from its outer boundary towards the centre. Such observations have also been recorded for other *Hepatozoon* species (Mackerras 1962; Ball et al. 1967, 1969; Bashtar et al. 1984). In the present study, the development of sporozoites in *H. mehlhorni* on day 16 p.i. was quite similar to previous findings in this genus (Mackerras 1962; Furman 1966; Ball et al. 1967, 1969; Bashtar et al. 1984, 1987).

Experimental transmission of the infectious stages of *H. mehlhorni* was accomplished by intraperitoneal inoculation of a homogenate of whole mosquitoes into uninfected vipers as previously described for *H. aegypti* and *H. gracilis* (Bashtar et al. 1984, 1987). This suggests that the natural mode of infection in the field would involve the transmission of infectious stages by mosquitoes. However, as another mode of transmission, Landau et al. (1972) have suggested that infectious stages of *H. domerguei* are transmitted after predation, e.g. during the ingestion of lizards by snakes.

References

Abdel-Ghaffar FA (1985) Light and electron microscope studies on a haemogregarine species infecting the viper *Cerastes vipera* in Egypt. Proc Zool Soc A R Egypt 9:209–220

Allison B, Desser SS (1981) Developmental stages of *Hepatozoon lygosomarum* (Dore, 1919) comb. n. (Protozoa, Haemogregarinidae), a parasite of a New Zealand skink, *Leiolopisma nigriplamtare*. J Parasitol 67:852–858

Anderson J (1898) Zoology of Egypt. Reptilia and Batrachia, vol I. Bernard Quaritch, London

Ayala SC (1970) A haemogregarine from sandfly infecting both lizards and snakes. J Parasitol 56:387–388

Ball GH, Oda SN (1971) Sexual stages in the life history of the haemogregarine *Hepatozoon rarefaciens* (Sambon and Seligmann, 1907). J Protozool 18:697–700

Ball GH, Chao J, Telford SR (1967) The life history of *Hepatozoon rarefaciens* (Sambon and Seligmann, 1907) from *Drymarchon corais* (Colubridae), and its experimental transfer to *Constrictor constrictor* (Boidae). J Parasitol 53:897–909

Ball GH, Chao J, Telford SR (1969) *Hepatozoon fusifex* sp. n., a haemogregarine from *Boa constrictor* producing marked morphological changes in the infected erythrocytes. J Parasitol 55:800–813

Barber DL, Mills-Westermann JE, Storoz P (1987) *Haemogregarina nototheniae*, new species from the blood of Antarctic notothenids. Syst Parasitol 10 (2):135–148

Bashtar A-R, Abdel-Ghaffar FA (1987) Light microscope study on the life cycle of *Haemogregarina najae* infecting the snake *Naja nigricollis nigricollis* (Elapidae, Proteroglypha, Squamata) from Egypt. Proc Zool Soc A R Egypt 14:33–44

Bashtar A-R, Boulos R, Mehlhorn H (1984) *Hepatozoon aegypti* nov. sp.: I. Life cycle. Z Parasitenkd 70:29–41

Bashtar A-R, Abdel-Ghaffar FA, Shazly MA (1987) Developmental stages of *Hepatozoon gracilis* (Wenyon 1909) comb. nov., a parasite of the Egyptian skink, *Mabuya quinquetaeniata*. Parasitol Res 73:507–514

Beyer TV (1977) Electron microscope study of *Karyolysus* sp. (Sporozoa: Adeleida; Haemogregarinidae) and of changes induced in the infected host cell. Protistologica XIII:57–66

Beyer TV (1982) Ultrastructural interrelation between lizard erythrocytes and haemogregarines (*Karyolysus*, Haemogregarinidae). Proceedings of the 5th International Congress on Parasitology (Toronto, 23–26 August 1982) p 98

Beyer TV, Scholtyseck E, Entzeroth R (1983) Fine structure of the merozoite of a haemogregarine from the testis of a lizard. Z Parasitenkd 69:439–445

Chatton E, Roubaud E (1913) Sporogonie d'une hemogregarine chez une tsetse (*Glossina palpalis* R. Desv.). Bull Soc Pathol Exot 6:226–233

Conceiçao-Silva FM, Abranches P, Silva-Pereira MCD, Janz JG (1988) Hepatozoonosis in foxes from Portugal. J Wildl Dis 24(2):344–347

Davidson WR, Calpin JP (1976) *Hepatozoon griseisciuri* infection in grey squirrels of the Southeastern U.S. J Wildl Dis 12:72–76

Elwasila M (1989) *Haemogregarina* sp. (Apicomplexa: Adeleorina) from the gecko *Tarentola annularis* in the Sudan: fine structure and life-cycle trials. Parasitol Res 75(6):444–448

Fantham HB, Porter A, Richardson LR (1942) Some haematozoa observed in vertebrates in eastern Canada. Parasitology 34:199–226

Furman DP (1966) *Hepatozoon balfouri* (Laveran, 1905): sporogonic cycle, pathogenesis, and transmission by mites to jerboa hosts. J Parasitol 52:373–382

Göbel E, Krampitz HE (1982) Histologische Untersuchungen zur Gamogonie und Sporogonie von *Hepatozoon erhardovae* in experimentell infizierten Rattenflöhen (*Xenopsylla cheopsis*). Z Parasitenkd 67:261–271

Hammond DM (1973) Life cycles and development of Coccidia. In: Hammond DM (ed) The Coccidia. University Park Press, Baltimore, pp 45–79

Hawking F, Perry W.L, Thurston JP (1948) Tissue forms of *Plasmodium cynomolgi*. Trans R Soc Trop Med Hyg 42:10–14

Hoare CA (1932) On protozoal blood parasites collected in Uganda with an account of the life cycle of the crocodile haemogregarines. Parasitology 24:210–224

Khan RA, Forrester DJ, Goodwin TM, Ross CA (1980) A haemogregarine from the American alligator *Alligator mississippiensis*. J Parasitol 66:324–328

Killick-Kendrick R (1984) Parasitic Protozoa of the blood of rodents: VI. Two new haemogregarines of the pygmy flying squirrel *Idiurus macrotis* (Rodentia: Theridomyomorpha: Anomaluridae) in West Africa. J Protozool 31:532–535

Kirmse P (1979) The ultrastructure of *Haemogregarina sachai* (Coccidia: Adeleidea) from the farmed marine flat fish *Scophthalmus maximus* L. Z Parasitenkd 58:201–210

Krampitz HE (1964) Über das Vorkommen und Verhalten von Haemococcidien der Gattung *Hepatozoon* Miller, 1908 (Protozoa, Adeleidea) in mittel- und südeuropäischen Säugern. Acta Trop (Basel) XXI:114–154

Landau I, Michel JC, Chabaud AG, Brygoo ER (1972) The life-history of *Hepatozoon domerguei*, comments on the fundamental characteristics of a coccidian life-cycle. Z Parasitenkd 38:250–270

Levine ND (1982) Some corrections in Haemogregarine (Apicomplexa: Protozoa) nomenclature. J Protozool 29:601–603

Lewis JE, Wagner ED (1964) *Hepatozoon sauromali* sp. n., a haemogregarine from the Chuckwalla (*Sauromalus* spp.) with notes on the life history. J Parasitol 50:11–14

Mackerras JM (1962) The life history of a *Hepatozoon* (Sporozoa: Adeleidea) of varanid lizards in Australia. Aust J Zool 10:35–44

Mehlhorn H (1988) Parasitology in focus: Facts and trends. Springer, Berlin Heidelberg New York

Michel JC (1973) *Hepatozoon mauritanicum* (Et. et Ed. Sergent, 1904) n. comb., parasite de *Testudo graeca*: redescription de la sporogonie chez *Hyalomma aegyptium* et de la schizogonie tissularie d'apres le matérial d'E. Brump. Ann Parasitol Hum Comp 48:11–21

Nadler SA, Miller JH (1984) A redescription of *Hepatozoon mocassini* (Laveran, 1902) n. comb. from *Agkistrodon piscivorus leucostoma* Troost, 1836. J Protozool 31:321–324

Paterson WB, Desser SS (1976) Observations on *Haemogregarina balli* sp. n. from the common snapping turtle, *Chelydra serpentina*. J Protozool 23:294–301

Robin LA (1936) Cycle evolutif d'un *Hepatozoon* de *Grecko verticillatus*. Ann Inst Pasteur 56:376–394

Shanavas KR, Ramachandran P (1990) Life-history of *Hepatozoon octosporei* sp. n., a new haemogregarine from the skink, *Mabuya carinata* (Schneider), with notes on the in vitro excystment of its oocysts. Arch Protistenkd 138:127–137

Sinha CK (1986) *Hepatozoon mucosus* sp. n. from Indian rat snakes, *Ptyas mucosus* (Linnaeus). Acta Protozool 25:471–476