Life cycle of *Hepatozoon affluomaloti* sp. n. (Apicomplexa: Haemogregarinidae) in crag lizards (Sauria: Cordylidae) and in culicine mosquitoes from South Africa

Johann Van As¹, Angela J. Davies²,³ and Nico J. Smit¹

¹Department of Zoology and Entomology, University of the Free State, Phuthaditjaba, South Africa;
²School of Life Sciences, Kingston University, Kingston upon Thames, United Kingdom;
³Water Research Group (Ecology), Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa

Abstract: A new haemogregarine species *Hepatozoon affluomaloti* sp. n. is described from erythrocytes in the peripheral blood of crag lizards *Pseudocordylus melanotus* (Smith) and *Pseudocordylus subviridis* (Smith) (Sauria: Cordylidae) from mountainous regions in the Eastern Free State, South Africa. This species can be distinguished from all other congeners based on its large size, staining properties and life cycle development in its vector, *Culex (Afroculex) lineata* (Theobald) (Diptera: Culicidae). Mature gamonts stain mostly uniformly pinkish-purple with Giemsa, sometimes containing darker azurophilic granules anterior and posterior to the nucleus. The reflexed posterior extremity of the gamont stage sometimes stains slightly deeper purple and the nucleus is dense and placed in the posterior third of the parasite body. Merogonic stages of this haemogregarine occur in the liver tissues of *P. melanotus* with dizoic meronts. Macromeronts contains 2–7 macromerozoites and micromeronts contains 9–24 micromerozoites. Sporogonic developmental stages found in the proposed final host and vector, *C. lineata*, include large oocysts, measuring 54 × 48 µm on average. Sporulating oocysts with 8 nuclei are present in mosquitoes 6–7 days post-feeding on infected lizards. Sporocysts with mature sporozoites measure 31.0 × 21.8 µm on average and each contains 2–8 large sporozoites. It is suggested that transmission of infective sporozoites is achieved through predation of lizards on mosquitoes.

Keywords: gamonts, merogonic stages, oocysts, sporocysts, sporozoites, transmission

Haemogregarines are usually recognised as apicomplexan blood parasites of vertebrates, with heteroxenous life cycles that involve one or more intermediate (vertebrate) hosts and haematophagous invertebrate final hosts (Bash-tar et al. 1987, Davies and Johnston 2000, Cook et al. 2009, Telford et al. 2012). Haemogregarines belong to several genera, with more than 30 species of the genus *Hepatozoon* Miller, 1906 having been described from lizards in Africa (Van As et al. 2013). *Species of Hepatozoon* are characterised by intraerythrocytic or intraleukocytic gamonts, but division stages are usually lacking in blood films. They also produce macro- and micromeronts typically in vertebrate host liver, and form polysporocystic oocysts in an arthropod invertebrate host (Smith 1996).

Most descriptions of *Hepatozoon* spp. are based on the morphology of the gamont stages in blood films, but Slo-boda et al. (2007) note that descriptions of species, without an account of a corresponding life cycle, are questionable. In particular, little work has been done to identify the final hosts of haemogregarines from lizards, i.e. usually haematophagous arthropods (Al Ghamdi et al. 2011, Telford et al. 2012), or their mode of transmission between hosts (Ball et al. 1967, Levine 1982, Bashtar et al. 1984, Telford et al. 2012). Landau (1973) suggested that transmission of species of *Hepatozoon* can be achieved by predation and this is likely in the case for the host lizards examined in this paper.

The present study thus provides description of a new species of *Hepatozoon* based on observation of its life cycle stages in lizards as well as in the invertebrate (final) hosts.

MATERIALS AND METHODS

Vertebrate host sampling and blood smear preparations

A total of 98 lizards were collected by hand (Free State Nature Conservation Permit Number: BBB002–00032–0035) during the summer months (September to April), over a period of 5 years (2008–2013). Sixty nine specimens of *Pseudocordylus melanotus* (Smith) were collected at Platberg reserve (28°14′36.71″S;
29°09′45.45″E) close to the town of Harrismith in the Eastern Free State, South Africa and 29 Pseudocordylus subviridis (Smith) were collected at the Sentinel trail (28°44′41.71″S; 28°53′05.56″E) in the North Eastern Drakensberg (Maloti mountains), Eastern Free State, South Africa.

A drop of peripheral blood was taken from each lizard, by toe clipping, and almost all lizards were released at their site of capture. Blood smears were made on clean microscope slides, air dried, fixed in absolute methanol for 1 minute and stored in dust-free boxes on site. Fixed slides were stained in the laboratory with a 9 : 1 solution of buffered Giemsa (Gurr® Improved R66 solution) for 30 min and scanned individually for parasites using a Nikon Eclipse photomicroscope (see Van As et al. 2013). Haemogregarine measurements and parasitaemia calculations were performed according to the methods in Van As et al. (2013). All measurements are in micrometres unless otherwise stated.

Maintenance of the vertebrate host, exposure to mosquitoes and subsequent sampling

One male of P. melannotus with a verified infection with the new species of Hepatozoon was kept in a terrarium for one week to acclimatise, provided with laboratory bred mealworms (Coleoptera: Tenebrionidae) and water. The lizard was then placed inside a 40 × 40 cm mosquito-rearing cage with newly emerged, uninfected female mosquitoes (see below). After the experiment the lizard was euthanised with 0.5 ml Euthapent (Kryon Laboratories, Johannesburg, South-Africa (edms) bpk) in 1ml tap water. Heart blood was taken with a micropipette immediately post mortem and small blocks of liver tissue were cut and smeared on clean microscope slides. Liver smears were fixed, stained and examined as for blood films.

Collection and sampling of mosquito hosts

Females of Culex lineata (Theobald) were collected in the field at night with an aspirator while feeding on P. melannotus that were residing in rock cracks. Engorged female mosquitoes were euthanised in ethyl acetate vapours sequentially from 1 to 30 days post feeding on a laboratory maintained lizard (above). The thorax, head and abdominal contents of the mosquitoes were then squashed between two microscope slides, similar to the methods described in Davies and Smit (2001) and Hayes et al. (2006) for blood-sucking crustaceans. Squashes were then fixed in absolute methanol for 1 min and stained in a 9 : 1 solution of buffered Giemsa for 30 min (as for blood films). Stained squash preparations were scanned for possible Hepatozoon developmental stages on a Zeiss stereomicroscope using the 40× objective. Photographs were taken with a Nikon Coolpix 4500 digital camera custom fitted to a Nikon Eclipse microscope.

Experimental mosquito hosts

Mosquito eggs and larvae of Culex (Culex) pipiens Linnaeus, Culiseta (Allotheobaldia) longiareolata (Macquart) and Culex andersoni hwamibani Edwards, all identified using the key in Jupp (1996), were collected from freshwater ponds in the town of Harrismith and were fed a 50 : 50 mixture of Nestum® (Nestlé®, Johannesburg, South Africa) and brewer’s yeast (Robertsons®, Cape Town, South Africa). Emerged adult female mosquitoes were kept alive at 25–30°C (50–60% RH) by feeding with 2% sucrose solution soaked cotton balls. The insects were then placed in a mosquito-rearing cage with P. melannotus specimen infected with the new species of Hepatozoon and were enticed to feed on the lizard by depriving them of the sucrose solution for 24 h.

RESULTS

Hepatozoon affluomaloti sp. n. Figs. 1–4

ZooBank number for species: urn:lsid:zoobank.org:act:FBAC258B-E6E5-47B4-820C-8EF6BCEE877F

Description of intraerythrocytic gamonts. Giemsa-stained blood films from Pseudocordylus melanotus, immature gamonts (Fig. 1A, thin arrow) rarely observed, forming parasitaemias of ~1/10000 (0.01%) of infected mature erythrocytes, measuring 11.0 × 3.5 (n = 1). Maturing or mature intraerythrocytic gamonts (Fig. 1B–D) from peripheral blood broadly elongate, sausage-shaped organisms with reflexed posterior pole (Fig. 1C,D; thin arrows) and broadly rounded, or pointed, anterior extremity (Fig. 1A,D,E–G; thick arrows). Infected erythrocytes with two gamonts (Fig. 1D) seen in blood films from one lizard. Gamont anterior pole with cap stained slightly deeper purple than remaining parasite body (Fig. 1D,E,H; thick arrows). Mature gamonts measured 15.8–21.8 (18.7 ± 1.4) long × 3.2–7.3 (5.7 ± 0.9) wide (n = 75). Gamont cytoplasm stained mostly uniformly, sometimes with darker azurophilic granules anterior and posterior to nucleus. Reflexed posterior extremity sometimes stained slightly deeper purple (Fig. 1C,D,E,H, thin arrows). Nucleus of gamont dense, placed in posterior third of parasite body, measuring 3.9–7.9 (5.8 ± 1.0) × 3.0–7.6 (5.0 ± 1.0) (n = 75).

Immature gamonts of H. affluomaloti sp. n. in blood of Pseudocordylus subviridis rarely observed forming parasitaemias of ~2/10000 (0.2%) of mature erythrocytes (Fig. 1K), measuring ~11.0 × 3.5 (n = 2). Gamont nuclei, staining dark pink with banded chromatin, covering more than half of parasite body; measuring ~6.0 × 3.5 (n = 2). Maturing and mature intraerythrocytic gamonts (Fig. 1L,M) elongated with rounded anterior extremity (Fig. 1M; thin arrow). Anterior pole broader than posterior pole, bearing anterior cap in some individuals (Fig. 1L; thin arrow). Gamonts measuring 17.4–22.0 (19.4 ± 1.0) × 5.4–7.6 (6.2 ± 0.7) (n = 60). Cytoplasm stained uniformly pinkish with Giemsa, sometimes with scattered granules anterior and posterior to nucleus. Slightly reflexed posterior end visible in some individuals (Fig. 1L,N, thick arrows). Dense, rounded to oval nucleus stained deep pinkish-purple, containing finely strangled or slightly granular chromatin, centrally or more posteriorly placed (Fig. 1L–N) in posterior third of parasite body, 5.0–10.1 (6.5 ± 0.9) long 4.0–6.1 (5.2 ± 0.5) wide (n = 60).

Description of gamonts in heart blood of Pseudocordylus melanotus

Gamonts of intermediate appearance between two types (Fig. 1E–G) observed in heart blood, as well as those identical to those in peripheral blood (Fig. 1H–J). Heart blood gamont stages elongated, with a rounded anterior extremity (Fig. 1F,G). Posterior pole strongly reflexed or sometimes
Fig. 1. Micrographs of intraerythrocytic gamont stages of *Hepatozoon affluomaloti* sp. n. from the peripheral and heart blood of *Pseudocordylus melanotus* and *P. subviridis*. **A–D** – maturing gamonts in the peripheral blood of *P. melanotus*; **E–G** – gamonts in the heart blood of *P. melanotus*; **H–J** – mature gamonts in the peripheral blood of *P. melanotus*; **K–N** – maturing gamonts in the peripheral blood of *P. melanotus*. **Thick arrows**: A,D,E–G,I – showing broadly rounded, or pointed, anterior extremity; D,E,H – deeper staining anterior pole; L,N – reflexed posterior pole. **Thin arrows**: A – immature gamont; C,D – reflexed posterior pole; C,D,E,H – deeper staining reflexed posterior pole; M – rounded anterior extremity; L – anterior cap; E – curved posterior pole.

curved (Fig. 1E, thin arrow) and occasionally stained darker than remaining cytoplasm of gamont. Host cell nucleus mostly less compacted than in peripheral blood stages, but still displaced laterally (Fig. 1E,F).

Occasionally, host cell nucleus absent from infected erythrocytes. Heart blood gamonts 18.7–21.0 (19.8 ± 0.9) long 3.1–4.2 (3.6 ± 0.5) wide (n = 20), tended to be longer and narrower than those in peripheral blood. Except at posterior pole, main cytoplasm of heart blood gamonts stained paler than its periphery, sometimes with dark azurophilic granules just anterior and posterior to nucleus (Fig. 1H–J). Dense nucleus stained dark purple-blue, situated centrally or in posterior third of parasite body, 5.7–8.0 (6.5 ± 0.8) long 2.2–3.8 (2.6 ± 0.5) wide (n = 7) (Fig. 1E–G).

**Description of merogonic stages in Pseudocordylus melanotus liver tissue**

Other internal organs (spleen, heart, kidneys and intestines) were also examined for merogonic stages, but none were observed. However, these organs are not ruled out as potential areas for development of this parasite as only a single lizard was studied.

Meronts were observed primarily in what were presumed to be hepatocytes or endothelial cells, and in the lizard specimen examined, no other organs harboured meronts. Young
meronts (Fig. 2B,C) presumably arose from sporozoites inoculated by vector and these stages measure 17.0–19.4 (18.3 ± 1.0) in length by 16.0–18.4 (17.1 ± 1.1) in width (n = 10) and apparently contain abundant amylopectin (Fig. 2C). Dizoic meronts (Fig. 2D) rare in liver tissue and measure about 16.8 × 16.2. Macromeronts (Fig. 2E,F) most abundant in liver smears measuring 25.9–33.9 (25.6 ± 2.3) in length by 20.1–26.7 (22.7 ± 2.3) µm in width (n = 10), with surface area of ~681.2 µm², containing 2–7 (4 ± 1.8) (n = 10) macromerozoites.

Individual macromerozoites within macromeronts measuring 15.8–22.4 (20.8 ± 1.8) in length by 2.9–8.5 (5.2 ± 1.7) in width (n = 10), similar in general morphology to gamont stages in peripheral blood, except that some are more pyriform in shape (Fig. 2E,F).

Macromerozoite cytoplasm stained whitish-blue with dark granules distributed randomly throughout. Nuclear periphery stained dark blue with center staining dark purple. Macromerozoite nuclei measured 2.8–6.2 (4.0 ± 1.0) by 1.9–3.1 (2.5 ± 0.4) (n = 10).

Fig. 2. Micrographs of merogonic developmental stages of *Hepatozoon affluomaloti* sp. n. in liver tissue of *Pseudocordylus melanotus.*

Ruptured macromeronts (Fig. 2G,H) release macromerozoites foamy in appearance and broader than those within meronts. Extracellular macromerozoites 18.4–22.0 (19.9 ± 1.1) in length by 7.2–13.0 (9.5 ± 1.3) in width (n = 13). Cytoplasm stained light whitish-pink with distinct pink granules distributed around nucleus. Elliptical nuclei (Fig. 2G,H) compact, stained deep purple, 3.6–7.0 (5.5 ± 0.8) in length by 2.6–6.6 (3.6 ± 1.1) in width (n = 13).

Micromeronts were also identified in lizard liver (Fig. 2I–K) and were presumed to arise from macromerozoites. Each micromeront produced 9–24 micromerozoites. Micromeronts generally slightly larger than mature macromeronts, 25–40 (33 ± 5.4) in length by 30.0–45.0 (38.5 ± 5.3) in width (n = 7), with surface area of 402.4–711.4 (506.9 ± 111.9) µm² (n = 10). Micromerozoites slender and more elongate than macromerozoites.
Fig. 4. Diagram representation of the life cycle of *Hepatozoon affluomaloti* sp. n. A – mature gamonts in the peripheral blood of *Pseu-
docordylus melanotus* are taken with a blood meal by female *Culex (Afruculex) lineata* mosquito; B, C – uninucleate and binucleate oocysts developed 3 days post feeding; D, E – sporocysts with developing sporozoites; F – sporocysts, containing maturing or mature sporozoites that resides in the gut contents; G – female *C. (A.) lineata* mosquito with mature sporozoites are ingested by *Pseudocordyl-
us melanotus* (H); I – L – young meronts in liver tissue of *P. melanotus* development to mature macromeronts with macromerozoites; M – macromeront ruptures and macromerozoites are released; N – macromerozoites re-infects the liver (O); P – mature micromeronts release micromerozoites (Q) that infect erythrocytes.

difficult to measure (none was extracellular) about 18 × 2.5 in size.

**Description of sporogonic stages in naturally feeding mosquitoes**

In squash preparations made from *C. lineata* 1–7 days post feeding (d.p.f) on infected lizard, haemogregarines and red blood cells (in different stages of digestion) from recent blood meal observed in gut contents (Fig. 3A–C). Morphometrically, ingested gamonts had similar dimensions to gamonts in erythrocytes of host lizard were rec-
nounced; two types: Encapsulated form (Fig. 3A) measured 10 × 2.6 (n = 1). Free gamonts long and slender (Fig. 3B,C), 21.4–28.4 (26.1 ± 2.1) in length by 3.7–5.6 (4.6 ± 0.6) in width (n = 10), with nuclei 6.6–13.2 (10.0 ± 1.8) in length by 3.1–5.7 (4.1 ± 0.8) in width (n = 10). Some free gamonts with broad anterior end(Fig. 3B), 22.9–26.8 (24.3 ± 1.9) in length by 4.7–6.4 (5.4 ± 0.6) in width a (n = 10). Nuclei of these broader gamonts 5.7–12.5 (9.3 ± 2.1) in length by 3.9–5.3 (4.5 ± 0.6) in width (n = 10).

Gametogenesis in naturally infected *C. lineata* and sub-
sequent fertilisation were not seen, but uninucleate and binucleate oocysts (Fig. 3D,E) present 3 d.p.f in the gut contents of mosquitoes measuring ~54 × 48 with area of ~2093 µm². Nuclei of oocysts measuring ~26.9 × 25.8 with area of ~694 µm². Six to seven days post feeding sporulating oocysts with 8 nuclei present in gut contents, measur-
ing ~25.5 × 26.1 with area of 666 µm², likely signalling the onset of sporogony (Fig. 3F), but mature polysporocystic oocysts not observed in these wild-feeding mosquitoes.

Sporocysts with developing sporozoites (Fig. 3G–I) measure 23.0–25.6 (24.1 ± 1.1) × 24.4–28.1 (26.1 ± 1.5) (n = 8) with average area of 632 µm². Sporozoites containing maturing or mature sporozoites (Fig. 3J–L) 26.7–39.2 (31.0 ± 3.8) in length by 17.0–25.6 (21.8 ± 0.6) in width, contained 2–8 (4 ± 1.4) large sporozoites (n = 20). Spo-
rozoe cytoplasm with foamy appearance and sporozoites 16.8–32.4 (22.4 ± 4.6) in length by 4.0–9.0 (5.1 ± 1.1) in width (n = 18). Each sporozoite nucleus rounded and dense
**Sporogonic stages in experimental mosquitoes (Culex and Culiseta spp.)**

Mature gamonts *H. affluomaloti* sp. n. were found in the gut of experimental mosquitoes (*Culex* Linnaeus and *Culiseta* spp.) one day post feeding on infected *P. melanotus*. Free gamonts were morphologically and morphometrically similar to those found gut contents of *C. lineata*, 21.4–28.4 (26.1 ± 2.1) in length by 3.7–5.6 (4.6 ± 0.6) in width (n = 10). Uninucleate and binucleate oocysts found in gut of *Culex pipiens* and *Culex andersoni* Edwards 3 days post feeding. Oocysts measuring ~51 × 44 with an area of ~2243 µm². Each oocyst nucleus measuring ~7.7 × 8.2 with area of ~64 µm². Oocyst-like structures also seen in gut contents of *Culiseta longiareolata*, they could not be identified with enough certainty to be included in this description. No further sporogonic developmental stages seen in these experimental mosquitoes.

**Effects on host erythrocytes**

In general, infected *P. melanotus* erythrocytes stained lighter than non-infected erythrocytes, but were neither hypertrophied nor dehaemoglobinised. However, in a blood film from one lizard specimen, infected red blood cells were dehaemoglobinised and a degree of cellular hypertrophy was observed (Fig. 1E–G). The host cell nucleus was usually elongated, compacted and displaced laterally (Fig. 1A,B,E,F,I), sometimes almost terminally (Fig. 1H), and occasionally fragmented (Fig. 1D,F). Parasitised host cell nuclei were larger in length and width and total surface area than uninfected erythrocyte nuclei (Table 1).

Infected erythrocytes in *P. subviridis* were generally not dehaemoglobinised but were paler stained and bigger than non-infected erythrocytes (see Table 1). The parasitised erythrocyte nucleus was compacted and displaced laterally (Fig. 1M) and was also larger in length, width and surface area (Table 1) than those of uninfected erythrocyte nuclei.

**Type host:** *Pseudocordylus melanotus* (Smith) (Sauria: Cordylidae).

**Other host:** *Pseudocordylus subviridis* (Smith).

**Type locality:** Platberg, Eastern Free State, 2101 m.

**Other localities:** Sentinel area, Northern Drakensberg (Maloti mountains), Eastern Free State, 2890–3050 m.

**Site of infection:** Peripheral blood.

**Other sites of infection:** Liver.

---

**Table 1.** Comparative measurements of surface area, length and width between infected and uninfected erythrocytes and their nuclei.

<table>
<thead>
<tr>
<th></th>
<th>Infected erythrocytes</th>
<th>Uninfected erythrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Erythrocyte</td>
<td>Erythrocyte nuclei</td>
</tr>
<tr>
<td>Host lizards</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudocordylus melanotus</em></td>
<td>SA (µm²)</td>
<td>length (µm)</td>
</tr>
<tr>
<td></td>
<td>161–223</td>
<td>6.4–12.3</td>
</tr>
<tr>
<td></td>
<td>(192 ± 21)</td>
<td>(9.1 ± 1.2)</td>
</tr>
<tr>
<td><em>Pseudocordylus subviridis</em></td>
<td>141–295</td>
<td>6.7–11.0</td>
</tr>
<tr>
<td></td>
<td>(210 ± 34)</td>
<td>(8.9 ± 0.9)</td>
</tr>
</tbody>
</table>

SA – surface area.

In appearance, measuring 1.8–5.6 (3.4 ± 1.0) by 1.9–4.6 (3.3 ± 0.7) (n = 18).

**Remarks.** The intraerythrocytic gamonts of *H. affluomaloti* are larger and wider than *H. langi*, *P. subviridis*, and *H. vacuolatus*. Although *P. subviridis* does occur sympatrically with *P. langi* at altitudes, their *Hepatozoon* infections are morphologically and morphometrically distinct. Gamonts of *H. langi* are encapsulated with narrow curved tails and measures 19.1 × 6.2 µm whereas *H. vacuolatus* has distinctive rounded and oval vacuoles and measures 16.5 × 5.9 µm. Gamont stages, similar in appearance to those occurring in the peripheral blood, were also observed in squash/smear preparations of *P. melanotus* liver tissue (Fig. 2A) and had presumably escaped the general circulation during tissue preparation. These extracellular gamonts were also morphometrically identical to the blood stream forms in Fig. 1A–J. In liver squash/smear preparations additional haemogregarine stages were observed.
oocyst stages were not stated by Bashtar et al. (1987), these stages were present in the haemocoel of the experimental mosquito host Culex p. molestus on day 5 to 8 post infection. Large oocysts of the new species (~51 × 44 µm with an area of ~ 2243 µm²) were seen in squashes of Culex p. and C. andersoni 3 days post feeding on H. affluomaloti infected blood. Subsequent dissections of these mosquitoes revealed only these oocysts and possible sporocytes, but no sporozoites were seen in these experimental mosquitoes. In wild specimens of Culex lineata collected while feeding on H. affluomaloti-infected lizards, oocysts of similar size as in experimental mosquitoes (~54 × 48 µm with an area of ~ 2093 µm²) were seen in the gut contents from 1–3 d.p.f. Elliptical sporulating sporocytes and subsequent stages with nuclear division were seen in C. lineata (Fig. 4G). Two C. lineata individuals also revealed sporocytes with developing sporozoites and these sporocytes (~24 × 26 µm) were approximately similar to those of H. gracilis (~22 × 19 µm) obtained from C. p. molestus.

Hepatozoon affluomaloti apparently differed from H. gracilis by having 2–8 large sporozoites per sporocyte that ranged from 16.8–32.4 µm in length by 4–9 µm in width, whereas H. gracilis produced more sporozoites (8–24) per sporocyte, but these are smaller ~10 × 2 µm. When compared to other lizard haemogregarines across Africa, H. affluomaloti overlaps in size with Hepatozoon mabuiae (Nicolle et Comte, 1906) in the scincid lizard, Trachylepis vittata (Oliver, 1804) from Tunisia, which measures 14–17 × 5–6 µm (see Table in Van As et al. 2013). When H. affluomaloti is compared with Hepatozoon gracilis Wenyon, 1909, which was redescribed by Van As et al. (2013) from another skink, Trachylepis quinquetaeniata (Lichtenstein), in the Sudan, gamonts of H. affluomaloti are much broader than those of H. gracilis, which are long and slender (18.0–22.2 × 0.9–1.4 µm); both species caused a slight degree of hypertrophy of host erythrocytes and lateral or sometimes terminal, displacement of host cell nuclei.

The merogonic stages of H. affluomaloti follow the same overall development pattern as recorded by Wenyon (1909) and Bashtar et al. (1987) for H. gracilis. Wenyon (1909) reported macromeronts with 8–16 macromerozoites in the liver of the lizard and macromeronts with an ‘enormous number’ of micromerozoites. Bashtar et al. (1987) reported ‘micromeronts’ that produced 3–16 macromerozoites and ‘macromeronts’ that produced up to 25–50 micromerozoites. Macromeronts of H. affluomaloti in liver tissue produced 2–7 macromerozoites, fewer overall than the macromerozoites from H. gracilis.

**DISCUSSION**

Hepatozoon affluomaloti sp. n. forms both immature and mature gamonts in the blood of Pseudocordylus melanotus and P. subviridis, but does not apparently divide in the erythrocytes nor causes their lysis. Observations of blood stages using light microscopy indicates, therefore, that it is unlikely a member of the genera Haemogregarina Danilewsky, 1885 or Karyolyssus Labbé, 1894. Squash/smear observations also demonstrate that it forms macro- and micromeronts in lizard liver tissue, suggesting it belongs to the genus Hepatozoon as defined by Smith (1996). The development of H. affluomaloti in natural and experimental mosquito hosts reported here further supports its placement in Hepatozoon base on criteria outlined by Smith (1996). Although gametogenesis and fertilisation were not observed in mosquitoes and mature, polypysogony oocysts were not detected, young oocysts with one, two and multiple nuclei were seen, and numerous sporocytes containing sporozoites were located in naturally infected mosquitoes found feeding on lizards with this haemogregarine.

Stages of H. affluomaloti in peripheral blood of P. melanotus and P. subviridis are morphologically and morphometrically alike in overall shape, and staining properties and measurements, except for a few additional granules in the cytoplasm of parasites from P. subviridis (Fig. 1K–N). Infections of H. affluomaloti also distorted infected erythrocytes in both lizard species to a similar extent. Therefore, it is assumed that both lizards are infected with the same haemogregarine.

Different variations in the life cycle patterns of Hepatozoon spp. have been reported (for review see Telford 2009). In the majority of species, the appearance of their gamonts in erythrocytes and/or leucocytes of vertebrate hosts, including reptiles, have been reported (Davies and Johnston 2000). Merogony does not usually occur within erythrocytes, but in vascular endothelial cells (Telford 2009). Latent monozoic and dixoic cysts can also exist in vertebrate tissues. In invertebrate hosts such as mites, ticks, insects and possibly leeches, microgametes may be flagellated, but no sporokinetes are formed. In the haemocoel of these same invertebrates, large polycystic oocysts are normally produced with sporocytes containing four to 16 or more sporozoites. Transmission occurs when the vertebrate host ingests the infected invertebrate or through predation on another vertebrate containing tissue cysts (Davies and Johnston 2000).

The range of blood sucking invertebrates that parasitise reptiles includes ixodid and argasid ticks, mites, assassin bugs, dipterans (sandflies, mosquitoes, tsetse flies), anoploleurotric arthropods, (sucking lice), siphonapterans ( fleas) and hirudines (leeches) (Smith 1996). Most life cycle studies have been carried out using mosquitoes as possible definitive hosts and Smith (1996) considers Culex, Aedes Meigen and Anopheles Meigen as the main vectors of species of Hepatozoon in ophidians. Low host specificity has been reported from members of this genus. For example Telford et al. (2004) reported Hepatozoon sauritus Telford, Wozniac et Butler, 2001 in four snake species of three genera. Ball (1967) observed in his experiments that Hepatozoon rarefaciens Sambon et Seligmann, 1907 is transferred from a colubrid snake (Drymarchon corais [Boie]) to a boa (Boa constrictor Linnaeus) by means of a mosquito (Culex tarsalis Coquillett). Other authors, e.g. Landau et al. (1970) and Paperna and Lainson (2004), have shown that Hepatozoon host specificity is even lower than that at the level of the first intermediate hosts, and found dixoic, tetraozoic or heaxazoic cysts in livers under experimental conditions.
Wozniak and Telford (1991) successfully transmitted a Hepatozoon species from two species of colubrid snakes, Coluber constrictor Stejneger et Barbour and Nerodia fasciata Linnaeus, to two species of Anolis Daudin. Smith et al. (1994, 1996) showed that even amphibians can serve as first intermediate hosts, at least under experimental conditions. Lowichik and Yaeger (1987) demonstrated experimentally using an ooviviparous snake, Nerodia fasciata, as an experimental host that congenital transmission can represent another route of infection for species of Hepatozoon.

Acknowledgements. This paper is dedicated to the memory of one of the authors (Angela J. Davies), who passed away in December 2013, i.e. in the final phase of the manuscript preparation. The financial assistance of the National Research Foundation (NRF; project IFR2011040100022) is acknowledged. Opinions expressed and conclusions arrived at are those of the authors and are not necessarily to be attributed to the NRF.

REFERENCES


Ball G.H., Chao J., Telford S.R. 1967: The life history of Hepatozoon rarefaciens (Sambon and Seligmann, 1907) from Dryamarchon corais (Colubridae), and its experimental transfer to Constrictor constrictor (Boidae). J. Parasitol. 53: 897–909.


Received 12 May 2014
Accepted 20 September 2014
Prepublished online 13 January 2015

Cite this article as: Van As J., Davies A.J., Smith N.J. 2015: Life cycle of Hepatozoon affluomaloti sp. n. (Apicomplexa: Haemogregarinidae) in crait lizards (Sauria: Cordylidae) and in culicine mosquitoes from South Africa. Folia Parasitol. 62: 008.