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**RESEARCH ARTICLE**

# Developmental stages of *Hepatozoon* sp. (Apicomplexa: Hepatozooidae) from Steudner's gecko, *Tropiocolotes steudneri* (Gekkonidae)

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## *Abstract*

One *Hepatozoon* sp. was detected from *Tropiocolotes steudneri* collected from Giza, Egypt. The parasite invaded only erythrocytes, sometimes they were extracellularly observed. Three different forms were detected: i) Small form with a size of 4.8–6.3×2.9–3.8 µm (L×W). ii) Intermediate form with a size of 7.6–9.4×3.2–4.5 µm (L×W). iii) Large form measured 12.4–17.3×5.1–6.2 µm (L×W). Merogony occurred only in endothelial cells of lung capillaries. Early and multinucleate meronts were seen. Micromeronts were subspherical to oval in shape, measuring about 17.5×15.7 µm and containing 2–5 macromerozoites. These merozoites were elongated, measuring 14.6×5.5 µm in an average size. Macromeronts were spherical to subspherical, measuring about 25.6×19.7 µm and containing 30–40 micromerozoites, measuring 10.7×3.5 µm in an average size.



## Introduction

Haemogregarines represent an important group of blood parasites, capable to infect all vertebrate groups. Four genera within this parasitic group are known to infect reptiles: *Haemogregarina* Danilewsky, 1885; *Karyolysus* Labbé, 1894; *Hepatozoon* Miller, 1908 and *Hemolivia* Petit, Landau, Baccam et Lainson, 1990. However, *Hepatozoon* is the most widely distributed genus among reptiles (Telford, 2009). The present investigation describes the erythrocytic as well as merogonic stages of *Hepatozoon* sp. parasitizing the Steudner's gecko, *Tropiocolotes steudneri* by light microscopy.

### MATERIALS AND METHODS

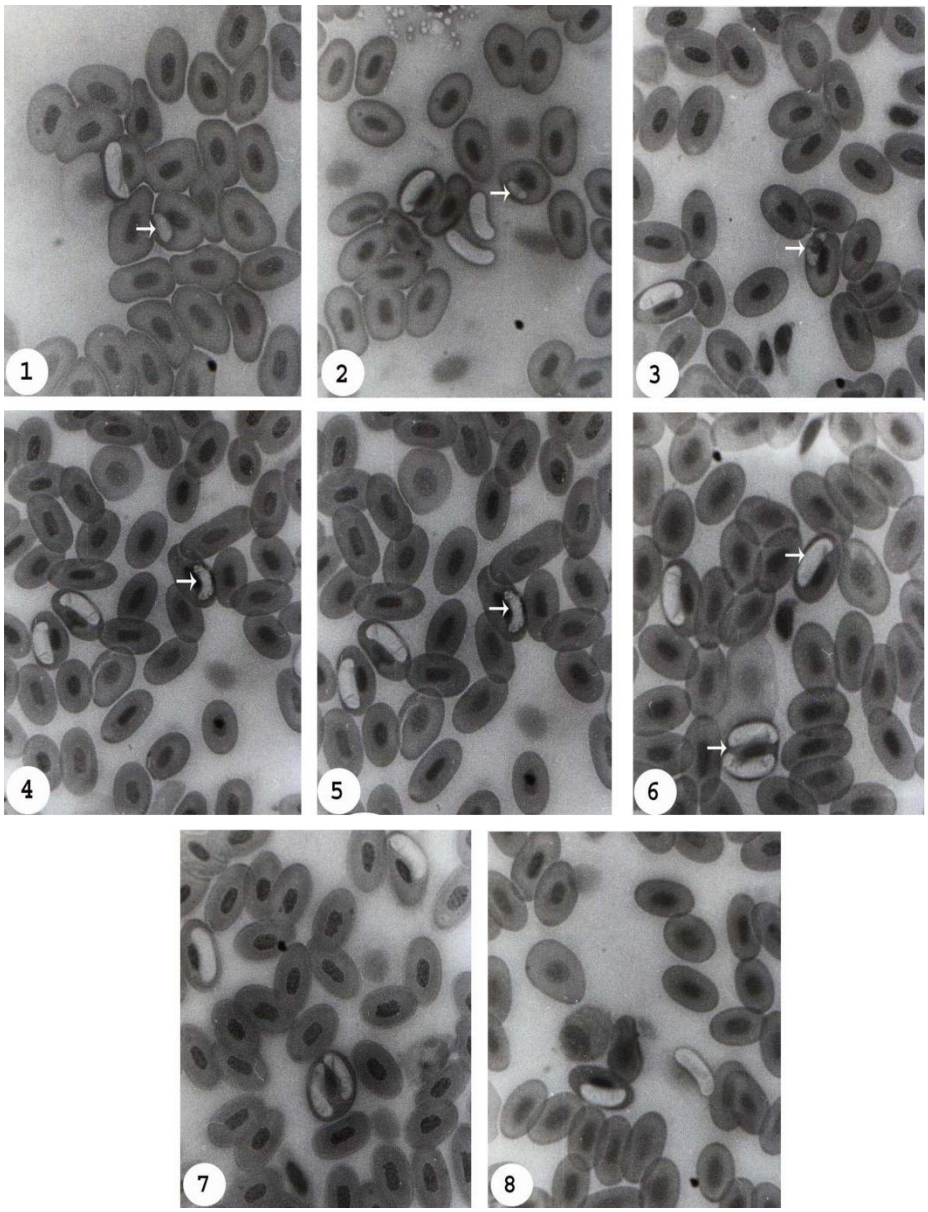
A total of seventeen geckos were collected from Abou Rawash, Giza, Egypt. Animals were brought alive to the laboratory and identified according to Saleh (1997). They were microscopically examined for blood and intestinal coccidian parasites. For blood parasites, thin blood films from liver, lung, heart, spleen and kidney of each gecko were prepared, air dried, fixed in absolute methanol and stained with 3% Giemsa. For studying the endogenous stages of the parasite, small pieces of lung, kidney, liver, spleen and heart of the positive specimens were immediately fixed in 70 % ethanol. Processing was done by the usual technique of dehydration in ascending series of alcohol, clearing in xylol and embedding in paraffin. Sections of 3–5 µm in thickness using a Rotary microtome were prepared and stained with haematoxylin and eosin. Finally, stained slides including that of thin blood films were microscopically examined and various developmental stages of the parasite were measured and photographed. For intestinal coccidian parasites, the alimentary canal of each gecko was removed and divided into segments. Wet smears from intestinal contents, gall bladder as well as kidney were immediately prepared and microscopically examined.

### RESULTS

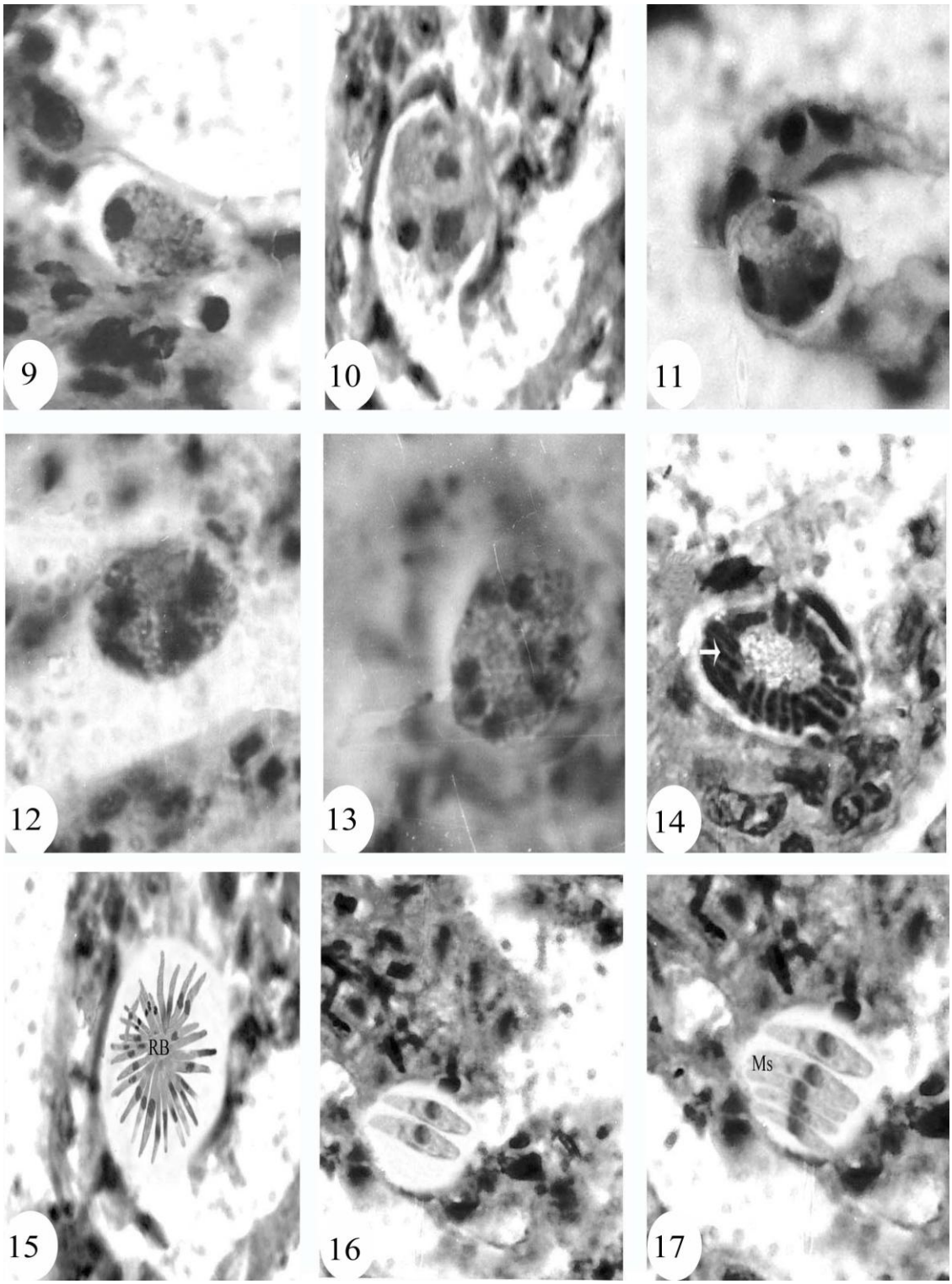
Only one out of seventeen geckos was found to be a natural host of only one *Hepatozoon* sp. None of any other blood or intestinal coccidian parasites were detected.

**Blood stages (Figs. 1–8)**

Blood stages of the parasite invaded only erythrocytes and none of the leucocytes were found to be parasitized. Sometimes, they were extracellularly observed (Figs. 2&8). Three different forms were detected: i) Small form (Figs. 1&2): It was oval, measuring about 4.8–6.3×2.9–3.8 µm L×W. ii) Intermediate form (Figs. 3–5): It had an eccentric nucleus, measured 7.6–9.4×3.2–4.5 µm L×W and considered as young gamont. iii) Large form (Figs. 1–8): It measured 12.4–17.3×5.1–6.2 µm. Nucleus of the infected erythrocyte was markedly displaced to the opposite side of the parasite. Double parasitic infection of a single host cell was observed (Figs. 6&7), the parasite in this case appeared more curved, especially in one side. Sometimes, one end of this form was rounded, while the other end was tapered and recurved.




**Figs. (1–8): Light micrographs of Giemsa-stained blood stages of *Hepatozoon* sp. naturally infecting *Tropiocolotes steudneri*. All photos x 2200**
**Figs. (1&2): Small forms. Figs. (3–5): Intermediate forms. Figs. (1–8): Large forms, the host cell nucleus was forced to the opposite side of the parasite. Figs (6&8): Double infections. Figs. (2&8): extracellular parasites. Note the parasite appeared more curved specially in one side.**




**Figs. (9–17): Light micrographs of merogonic stages in endothelial cells of lung capillaries (Haematoxylin–eosin stained sections). All photos x 2400**
**(Fig. 9): Early meront. Figs. (10–13): Multinucleate meronts. Fig. (14): Beginning of the budding of developing merozoites as finger–like outgrowths from the outer border of a macromeront. Fig. (15): A macromeront with micromerozoites still attached to the residual body. Figs. (16&17): Micromeronts with fully formed macromerozoites each merogonic stages was enclosed a parasitophorous vacuole.**

**Merogony and merozoites (Figs. 9–17)**

Merogony occured only in endothelial cells of lung capillaries. None of the merogonic stages were observed in circulating blood, or in any other organs. Early meronts were subspherical to ovoid, measuring about 10.3×8.4 µm (Fig. 9) and multinucleate once measured about 18.4×13.3µm (Figs. 10–13).

Merozoites appeared as finger–like outgrowths on the surface of meronts (Fig. 14). Two types of meronts were recognized. Micromeronts were subspherical to oval in shape, measuring 17.5×15.7 µm in an average size and containing 2–5 macromerozoites (Figs. 16&17). The latter were elongated, measuring about 14.6×5.5 µm (Figs. 16&17). Macromeronts were spherical to subspherical, measuring about 25.6×19.7 µm in an average size and containing 30–40 micromerozoites (Fig. 15), each one measured 10.7×3.5 µm (Fig. 15) in an average size each merongonic stages was enclosed a parasitophorous vacuole.

**DISCUSSION**

Satisfactory identification of a certain species of haemogregarines often proves to be one of the most difficult tasks the protozoologist may ever meet (Mohammed and Mansour, 1960). Smith and Desser (1997) concluded also that the systematic of haemogregarines is far from being resolved. Generic identification of haemogregarines is based on some criteria such as characteristics of blood forms, merogonic stages, vertebrate and invertebrate hosts and characteristics of sporogonic cycle. The latter is an important criterion used to differentiate between the genera. However, the vectors and details of sporogonic cycle are unknown for the majority of haemogregarines. So, the designation of a haemogregarine to any genus is difficult.

Siddall (1995) stated that "every parasite of lizards, snakes, crocodilians and birds that was originally described as a species of *Haemogregarina*, and for which sporogonic development has subsequently been discovered, has multisporocystic oocysts and has been transferred to genus *Hepatozoon* (e.g. Pessôa, 1970; Pessôa *et al*., 1970, 1972; Baker *et al*., 1972; Michel, 1973 and Naddler and Miller, 1984), thus, all remaining species of *Haemogregarina* described from the previously mentioned animal groups (lizards, snakes, crocodilians and birds) should be transferred to genus *Hepatozoon*". The systematic review of the haemogregarine complex, carried out by Smith (1996) has also resulted in the expansion of genus *Hepatozoon* to include all members of genus *Haemogregarina* that infect all groups of tetrapod vertebrates. So, he transferred a total of 203 species of *Haemogregarina* (*sensu lato*) to the genus *Hepatozoon*. These included 163 species of reptiles (95 from snakes). Moreover, Smith also transferred 2 species of *Haemogregarina* (*sensu stricto*), namely *H. algiri* and *H. cantliei* to genus *Hepatozoon*, as they are in fact parasites of snakes, not of turtles. Later, other authors also transferred several *Haemogregarina* spp. infecting some reptiles to *Hepatozoon* spp. (e.g. Telford *et al*., 2002a; Paperna and Lainson, 2004). Furthermore, haemogregarines infecting snakes, the complete life cycle of which are known, were also found belonging to the genus *Hepatozoon* (e.g. Telford *et al*., 2001; 2002a; 2002b). So, some authors based their identification of haemogregarines after Siddall (1995) and Smith (1996) on only the developmental stages inside the vertebrate host (Abdel–Gawad *et al*., 2002; Shazly, 2003; Abou El–Nour, 2005 and Abdel–Aziz *et al*., 2010).

Considering the above mentioned discussion, the present coccidian was placed into genus *Hepatozoon* along with many other haemogregarine species infecting snakes and lizards. However, it is also very important to study the vector and sporogonic cycle of such haemogregarines including the present one.

Views differ regarding the host specificity in haemogregarines, Levine (1982) concluded that some haemogregarines have a wide host range in both vertebrates and invertebrates, whereas others apparently do not. Elwasila (1989) suspected the presence of a sort of host specificity in haemogregarines and added that, this may explain the difficult of identifying the proper invertebrate hosts of these parasites. Mohiuddin *et al*. (1967) concluded that "the pattern of classification followed in most cases, has been to consider that each reptilian host has its own species of haemogregarine".

On the other side, specific identification of haemogregarines has also been rather unsatisfactory because of insufficient knowledge of their life histories (Mohiuddin *et al*., 1967). It is also based on some criteria such as morphological characteristics and measurements of blood forms, effect of the parasite on both host cells and their nuclei, the host and geographical distribution.

Primarily, the distinctive characters of the current parasite showed the common characteristics of many other haemogregarines as follows: (1) Blood stages invaded only the erythrocytes, which were hypertrophied and showing

**Table (1): Comparative data of haemogregarines from geckkonid hosts including the present one**

| Species of haemogregarine | Host | No. forms blood stages | Size of gamont (µm) | | Size of gamont's nucleus (µm) | | Site of merogonic stages | Size of mature meronts in average (µm) | | No. micro-merozoites in macromeront | No. macro-merozoites in micro-meront | Locality | Author (s) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Length | Width | Length | Width | | Micro- | Macro- | | | | |
| *Haemogregarina* sp. | *Ptyodactylus lobatus* | No data | Short | No data | No data | No data | No data | No data | No data | No data | No data | Egypt | Plimmer (1912) |
| *Haemogregarina* sp. | *Tarentola annularis* | No data | Short - bulky | No data | No data | No data | No data | No data | No data | No data | No data | | |
| *Haemogregarina* sp. | *Tarentola mauritanica* | No data | No data | No data | No data | No data | No data | No data | No data | No data | No data | Mediterranean | |
| *Haemogregarina platydactyli* | *Tarentola mauritanica* | 3 | 14.0-16.0 | 6.0-7.0 | No data | No data | Lung & Liver | 12.0 × 17.0 | 18.0 × 36.0 | No data | 8 | Algeria | Foley & Catanei (1925) |
| *Hepatozoon burneti* | *Tarentola mauritanica* | 3 | 35.0 | 6.0 | No data | No data | Lung & Liver | No data | No data | 10 - 20 | 10 - 20 | Tunisia | Lavier & Callot (1938) |
| *Haemogregarina* sp. | *Gehyra variegata* | _ | 11.0-14.0 | 4.0-6.0 | No data | No data | Lung | No data | No data | No data | No data | Australia | Stehbens & Johnston (1968) |
| *Haemogregarina* sp. | *Tarentola annularis* | 3 | 12.4 - 15.9 | 3.2-5.5 | No data | No data | No data | No data | No data | No data | No data | Sudan | Saoud & Younis (1969) |
| *Haemogregarina* sp. | *Tarentola annularis* | 1 | 12.5 | 3.6 | No data | No data | Lung, Liver & Spleen | 12.0 × 14.0 | 12.0 × 16.0 | 16 - 25 | 1 - 5 | Sudan | Elwasila (1989) |
| *Haemogregarina* sp. | *Ptyodactylus hasselquisti* | 2 | 24.3 | 8.5 | No data | No data | Lung | No data | No data | 14 - 20 | 4 - 8 | Egypt | Abdel Ghaffar *et al.* (1994) |

Table (1): Cont.

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Haemogregarina tarentannulari* | *Tarentola annularis* | 3 | 13.0-17.0 | 2.5-3.5 | 5.0-6.0 | 2.5-3.5 | Lung | 28-34 13-17 | 22-29 15-21 | 16 | 27-35 | Egypt | Saoud *et al.*(1995) |
| *Haemogregarina rawashi* | *Ptychodactylus hasselquisti* | 3 | 14.0-20.0 | 3.5-5 | 8.3 | 5.0 | No data | No data | No data | No data | No data | | |
| *Haemogregarina helmymohammedi* | *Hemidactylus flaviviridis* | 3 | 17.5-20.7 | 3.0-4.5 | 11.0-18.0 | 3-4.5.0 | Liver | 16 ×10 | 22 ×13 | 21 | 6 | | |
| *Haemogregarina tarentannulari* | *Tarentola annularis* | 3 | 13.0-17.0 | 2.5-3.5 | No data | No data | Lung & Liver | 28-34 ×19-25 | 30-40 ×15-20 | 40 | 15 | Egypt | Mohammed & Ramadan (1996) |
| *Haemogregarina rawashi* | *Ptyodactylus hasselquisti* | 3 | 14.0-20.0 | 3.5-5.0 | No data | No data | Lung & Liver | 28-35 ×25-31 | 22-28 ×15-20 | 37 | 16 | | |
| Two haemogregarines | *Ptyodactylus hasselquisti* | 2 | 22.0 | 10.0 | No data | No data | Lung | 13.9 ×10.1 | 20.9 ×17.6 | 12 | 24 | Saudi Arabia | Ahmed *et al.* (1999) |
| *Hepatozoon* sp.$_2$ | *Tarentola annularis* | 3 | 15.8-18.3 | 6.2-7.0 | No data | No data | Lung | 18.6 × 14.5 | 24.9 × 17.7 | 17 - 33 | 4 - 14 | Egypt | Abou El–Nour, 2005 |
| A haemogregarine | *Ptyodactylus hasselquistii* | 2 | 12.2-19.4 | 6.12-12.2 | No data | No data | Lung | 14.9 ×13.1 | 26.3 ×16.2 | 2 - 6 | 8 - 14 | Egypt | Hussein, 2006 |
| *Hepatozoon* sp.$_1$ | *Tarentola mauritanica* | 3 | 14.6-16.5 | 4.8-6.0 | 3.0 | 0 | Lung | 15.4 ×11.6 | 23.5 ×16.8 | 11 - 25 | 3 - 8 | Egypt | Abdel Aziz *et al*., 2010 |
| *Hepatozoon* sp. | *Tropiocolotes steudneri* | 3 | 12.4 – 17.3 | 5.1 – 6.2 | No data | No data | Lung | 17.5 × 15.7 | 25.6 × 19.7 | 30 – 40 | 2 – 5 | Egypt | The present study |

deformation. (2) The different forms of blood stages had no effect on the host cell nuclei. (3) Presence of two distinct types of meronts: micro- and macromeronts which yielded a few number of macromerozoites and a large number of micromerozoites, respectively. (4) Presence of a parasitophorous vacuole enclosing each developmental stage.

By comparing the data of the current parasite with that of the other haemogregarines previously described from gekkonid hosts (Table 1), It was found that, there were no obvious differences. So, the present haemogregarine was considered *Hepatozoon* sp.

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