

A DESCRIPTION OF *HAEMOGREGARINA* SPECIES NATURALLY INFECTING WHITE-SPOTTED GECKO (*TARENTOLA ANNULARIS*) IN QENA, EGYPT

By

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Abstract

The present study describes the developmental stages of *Haemogregarina* species in the blood and tissues of naturally infecting white-spotted gecko *Tarentola annularis* collected from Qena, Egypt. Different parasite's forms were observed infecting the erythrocytes. The gamonts enclosed within parasitophorous vacuole and seems to have no clear effect on the host cell especially in case of immature parasite forms. But in the presence of mature gamonts the host cell nucleus displaced. The parasitaemia level is up to 280 per 10,000 erythrocytes counted. Trophozoites and gamonts have been recognized in the blood smears. The rounded trophozoite diameter is $3.84 \pm 0.87 \mu\text{m}$, while the elongated trophozoite measured $4.42 \pm 0.69 \times 2.8 \pm 0.56 \mu\text{m}$. The mature gamonts were differentiated into two forms; short gamont measuring $10.82 \pm 0.82 \times 3.30 \pm 0.73 \mu\text{m}$ (range: $10-12.1 \times 2.2-4.4 \mu\text{m}$) and the long gamont measured $14.67 \pm 0.83 \times 3.96 \pm 0.77 \mu\text{m}$ (range: $14.1-16.5 \times 3.3-5.5 \mu\text{m}$). Merogony carried out only in the endothelial cells of the blood capillaries in the lung. Different merogonic stages have been recognized and differentiated in two forms; micromeront measured $13.25 \pm 0.50 \times 12 \pm 0.0 \mu\text{m}$ and produces a few number of large merozoites, macromeront measured $19.75 \pm 0.87 \times 13.25 \pm 0.50 \mu\text{m}$ and produces more small sized merozoites. The gamonts and merozoites have the general characteristic ultrastructures of the Apicomplexa containing components of the apical complex, e.g. pellicle, micronemes, rhoptries, and few dense bodies and subpellicular microtubules.

Key words: *Haemogregarina*, *Tarentola annularis*, Parasitaemia, Light and Electron microscope, Egypt.

Introduction

Reptiles are very old in the evolutionary scale and their parasites seem to become stabilized all over the world. Parasitism causes deleterious effects on several aspects of the ecology and evolution of hosts (Shazly 2000; Smallridge and Bull, 2000; Eisen, 2001; Amo *et al*, 2005).

Plimmer (1912) reported haemogregarines in *Tarentola (T.) annularis* and *Ptyodactylus (P.) lobatus* from Egypt. Several records of haemogregarines from reptiles in various parts of the world are known (Chatton and Blanc 1914; Foley and Catanei 1925; Saoud and Younis 1969; El-Wasila, 1989; Shazly 2000; Amo *et al*, 2004; 2005; Bouma *et al*, 2007; Al-Farraj 2008; Mihalca *et al*, 2008; Cook *et al*, 2009; Abdel-Baki and Al-Quraishy, 2012; El-Toukhy *et al*, 2013; Abdel-Haleem *et al*, 2013).

Haemogregarines infecting *T. annularis* were studied by many authors (El-Naffar *et al*, 1991; Ramadan *et al*, 1995; Saoud *et al*, 1995; Mohammed and Ramadan, 1996; Rabie *et al*, 2006; Abd-Al Aal *et al*, 2009). *Haemogregarina* species infecting the geckoes *P. hasselquistii* were also studied (Abdel-Ghaffar *et al*, 1994; Saoud *et al*, 1995; Hussein, 2006). Martínez-de La Puente and Merino (2008) supported the hypotheses proposed that the multiple invasions being due to higher parasite densities. The parasitaemia level of haemogregarines infecting *Mabuya (M.) quinquetaeniata* was 1%-8% and in some cases 40%-50% of the erythrocytes (Bashtar *et al*, 1987). While in *P. hasselquistii* infected with haemogregarine (*Karyolysus*) was up to 410/10,000 counted erythrocytes (Hussein, 2006). The parasitaemia level was 200-400/1000 erythrocytes of haemogregarines infecting

Cerastes c. gasperitti (Al-Farraj, 2008). In *M. quinquetaeniata* parasitized by *Hepatozoon gracilis* the level was slight ($\leq 2\%$), moderate (3-10%) and high (11-20%) per 1000 counted erythrocytes (Galal, 2010). Electron microscopy was used in studying blood and merogonic stages of haemogregarines in reptiles (Abdel-Ghaffar *et al*, 1994; Desser 1997; Smallridge and Paperna, 2000; Jovani *et al*, 2004; Rabie *et al*, 2006; Hussein, 2006).

The present study aimed to renew knowledge on the blood and tissue stages of haemogregarines infecting the Egyptian gecko, *T. annularis*.

Materials and Methods

Geckoes were handily captured and maintained separately in cages with suitable food and enough water at room temperature (28-35°C). Blood films were made, stained in Giemsa and examined by light microscope. From the highly infected animals, small pieces (2 mm) of lung and liver were washed in 0.9% physiological saline solution, fixed in 3% glutaraldehyde buffered in 0.1 M sodium cacodylate (pH 7.3), then washed in the same buffer, postfixed in 2% osmium tetroxide, washed in the buffer and dehydrated in ethanol. The specimens were embedded in araldite. Semithin sections were made on a Reichert microtome (thickness 0.5-0.7 μ m), stained using methylene blue and examined by a Leica research photomicroscope. Ultrathin sections (40-50nm) were made and stained and contrasted using lead citrate and uranyl acetate (Reynolds, 1963) and then examined with a JEOL transmission electron microscope (JEM-1010). The blood and tissue stages (from the semithin section) were observed and photographed using a Leica DM1000 microscope with a Leica EC3 camera. Measurements were done with an eyepiece micrometer with an oil-immersion lens, and given in micrometers as: mean \pm standard deviation followed by the range in parentheses. The present data have been compared with those previously described (Tab. 1).

Results

Blood stages and parasitaemia: In blood smears of infected *T. annularis*, different forms of the blood stages were observed infecting the erythrocytes and none of the leucocytes are infected. The parasites are usually intracellular. The infected erythrocytes were not affected by the presence of parasites. The immature blood stages of parasite have no effect on the host cell nucleus, but in case of infection with mature gamont the host cell nucleus had been displaced. Also the parasites have no karyolytic effect (Figs. 1&2). The parasitophorous vacuole surrounding blood and tissue stages were recognized using light microscope around some parasite stages (Fig. 3). Only single gamont and very rarely double gamonts were observed in the infected erythrocyte (Fig. 4). The gamont did not attack leucocytes. No fin-folds were seen within the gamont. The parasitaemia level (number of parasite/10.000 erythrocytes) was up to 280/10,000 erythrocytes. Host cell seemed to be not highly affected, where the infected cells measured $15.18 \pm 1.78 \times 10.56 \pm 0.77 \mu$ m (range: 13.2-17.6 \times 9.9-11.0 μ m) and the uninfected one measured $16.80 \pm 1.53 \times 10.12 \pm 0.70 \mu$ m (range: 14.0-18.7 \times 9.9-8.8 μ m) in length and width, respectively.

In blood smears, trophozoites and gamonts were recognized. The trophozoites appears firstly rounded and then elongated (Figs. 1&2). The rounded form diameter measured $3.84 \pm 0.87 \mu$ m (range: 3-4.4 μ m), while the elongated trophozoite measured $4.42 \pm 0.69 \times 2.8 \pm 0.56 \mu$ m (range: 3.3-5 \times 2.2-3 μ m). The mature gamonts (Figs. 2&3) were differentiated into two forms; short gamont measuring $10.82 \pm 0.82 \times 3.30 \pm 0.73 \mu$ m (range: 10-12.1 \times 2.2-4.4 μ m) and the long gamont measured $14.67 \pm 0.83 \times 3.96 \pm 0.77 \mu$ m (range: 14.1-16.5 \times 3.3-5.5 μ m).

Merogony and merozoites: Meronts in different developmental phases were seen - in few numbers - in the endothelial cells of the blood capillaries of the lung (Figs. 5-8).

But, none was seen in parenchyma cells of infected geckos' liver.

Merogony begins by the invasion of the parasite (sporozoite) to the endothelial cells of the blood vessels in the lung. The parasite became more rounded and began to grow in size forming developing meronts. The parasitophorous vacuole appeared enclosing the parasite and grows in size during the growth of the meront. The formation of merozoites began with the division of parasite nucleus into nuclei. Few daughter nuclei were seen peripherally arranged in the meront (Figs. 5-7). The cytoplasm remained, after the separation of the merozoites, appeared as a residual body (Fig. 7). The nuclei of the multinucleated meront undergo elongation and finally formed merozoites (Figs. 7&8). There were different merogonic stages; mononucleated meront (Fig. 5) and polynucleated meront (Figs. 6-8). According the measurements, the meronts are present in two forms; the small form (micromeront) measured $13.25 \pm 0.50 \times 12 \pm 0.0 \mu\text{m}$ and produces a few number of large merozoites (Figs. 5&6). The large form (macromeront) measured $19.75 \pm 0.87 \times 13.25 \pm 0.50 \mu\text{m}$ and produces more small sized merozoites (Figs. 7&8).

By the ultrastructure study, the gamonts and merozoites have the general characteristic ultrastructures of the Apicomplexa; containing components of the apical complex, e.g. pellicle, micronemes, rhoptries, very few dense bodies and subpellicular microtubules, which were located mainly at one pole of the parasite. Also residual body had seen after the merozoites formation (Fig. 9).

Discussion

Haemogregarines are apicomplexan protozoa belong to a group of blood protozoa, in which gamonts infected blood cells of reptiles and some other vertebrates. Merogony takes place in certain internal organs of the vertebrate hosts whilst sporogony occurs in invertebrate hosts (Levine 1982). Classification of haemogregarines depends on many criteria as site of schizogony and mode of

transmission after the specific sporogonic process within its haematophagus vector as well as its host range (Bashtar *et al.*, 1984; Ahmed 1998; Shazly, 2000).

In present study, no marked effect was induced by the parasite to the infected erythrocyte. Also the host cell nucleus didn't affected neither in position nor its shape or structure due to immature parasite stages occurrence, but the host cell nucleus has displaced in case of presence of mature gamonts. This result partially disagreed with Saoud *et al.* (1995), where trophozoite and gametocyte stages of *H. helmymohammedi* caused the erythrocytes to be hypertrophied, stretched and stain paler than the uninfected ones. Also, the host cell nucleus usually displaced by parasites, and also, disagreed with the study carried out by Al-Farraj (2008) where infected erythrocytes were hypertrophied and elongated. Hussein (2006) and Abdel-Haleem (2013) reported that the infected erythrocytes were hypertrophied with their nuclei either longitudinally stretched or split into two fragments. The present data agreed with Saoud *et al.* (1995) in case of *H. rawashi* Mohammed and Ramadan 1996 on only displacement the host cell nucleus by gamonts, but disagreed in case of immature gamont presence.

Regarding intensity of infection, nearly all studies included the parasitaemia level, but very few discussed its importance. About the effect of parasitaemia of *Hepatozoon gracilis* infecting *M. quinquetaeniata* on some biological aspects of vertebrate and invertebrate hosts, Galal (2010) observed that the daily mortality rate increased gradually with the increase in parasitaemia level. Also, Hussein (2006) reported the parasitaemia level in case of *Karyolysus* sp. infected *P. hasselquisti*, but didn't refer to parasitaemia effect on hosts. Bashtar *et al.* (1987) considered the parasitaemia (1%-8%) in skinks was high and in some cases 40%-50% of the erythrocytes were parasitized. The present study reported a moderate level of parasitaemia (280/10.000).

Abdel-Ghaffar *et al.* (1994) differentiated the gamonts of *Haemogregarina* sp. infecting *P. hasselquisti* into two forms; small meront measured 10×5 µm, while and the second form was large, measured 24×9 µm and represented the mature gamont. Saoud *et al.* (1995) described *H. helmymohammedi* as new species infecting *Hemidactylus flaviviridis* and redescribed *H. rawashi* Mohamed and Ramadan, 1996 infecting *P. hasselquisti* and *H. tarentanulari* parasitizing *T. annularia*. These data show differences with the data of the present materials. Rabie *et al.* (2006) described a haemogregarine species from *P. hasselquisti*, which had two forms of blood stages; short measured 8×4 µm and the large gamont measured 13×4 µm. The merogont occurred in the host's lung and liver. The merogony stages were differentiated into two forms; micromeront measured 12×9 µm and macromeront 20×13 µm. The present forms of the gamonts and meronts were different by measurements and merogony was found only in lung.

The present gamonts were seemly close to the gamonts of haemogregarine species (*Karyolysus*) described by Hussein (2006), but infecting different host, *P. hasselquisti*, and also the previously parasite had a karyolytic effect on the host cell nucleus. The present gamonts have no karyolytic effect on the host cell nucleus. Comparison of host, geographical distribution and measurements between the present species and the previously described *Haemogregarina* species in geckoes from different localities in Egypt revealed that the present *Haemogregarina* species infection *T. annularis* was not identical to any of those previously described.

Regarding the merogony in the previously mentioned parasites were found in lung and liver. Merogony was observed only in the endothelial cells of the blood capillaries in the lung only of *P. hasselquisti* (Abdel-Ghaffar *et al.*, 1994). In the present study, merogony was found only in lung and the different measurements of the present tissue stages differed from those previously de-

scribed. The cyst forming coccidians maintained their 3-layered pellicle during schizogony (Abdel-Ghaffar *et al.*, 1994). The schizont's nucleus eventually underwent repeated divisions and the resulting daughter nuclei migrated to the periphery of the schizont directly underneath the outer boundary. The first indication of merozoite formation was a thickening and protrusion of the schizont boundary at sites where a nucleus was close to this membrane and this structure is termed merozoite anlage. Similar observations were reported on the coccidia as well as the subsequent development and formation of merozoites, where such process was described as ectomerogony (Bashtar *et al.*, 1984; Shazly, 2000). The fine structure of the merozoite was similar to that described for the erythrocytic stages of the parasite, except for the presence of more amylopectin granules in the merozoites. This was observed in many coccidian merozoites (Bashtar *et al.*, 1984; Ahmed, 1998; Shazly, 2000; Mehlhorn, 2001). The cell boundary of the erythrocytic gamonts and meronts are consisted of an outer unit membrane and an inner complex of 2 closely opposed unit membranes (Abdel-Ghaffar *et al.*, 1994; Hussein, 2006). In the present material, the ultrastructure showed that each parasite was covered by a pellicle consisting of two layers, (but not easy to recognize if the inner membrane complex seen to resolve into two unit membranes). The present study showed that the gamont had the structural elements of the apicomplexa as the two membranes pellicle, subpellicular microtubules, rhoptries, homogenously distributed micronemes and some of dense bodies were distributed in the cytoplasm. Also the daughter nuclei had been peripherally arranged.

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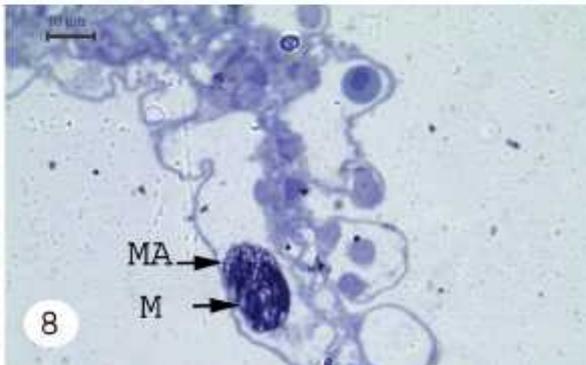
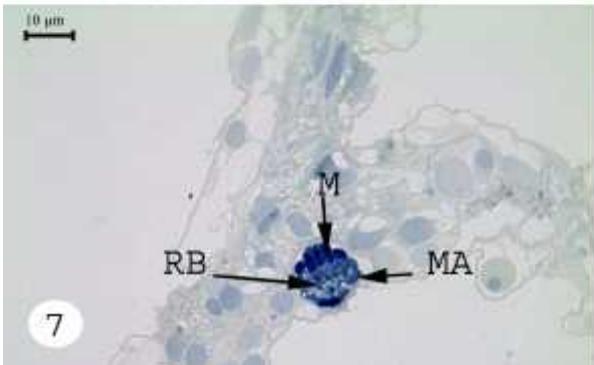
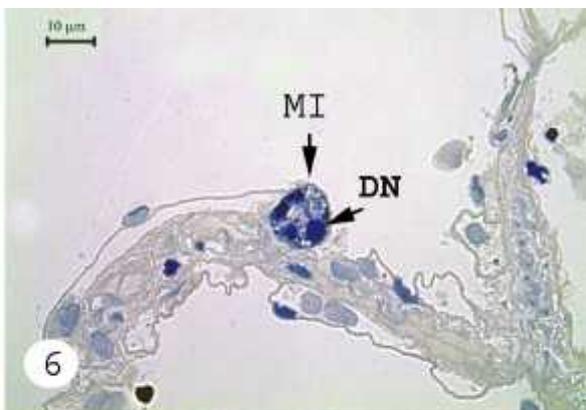
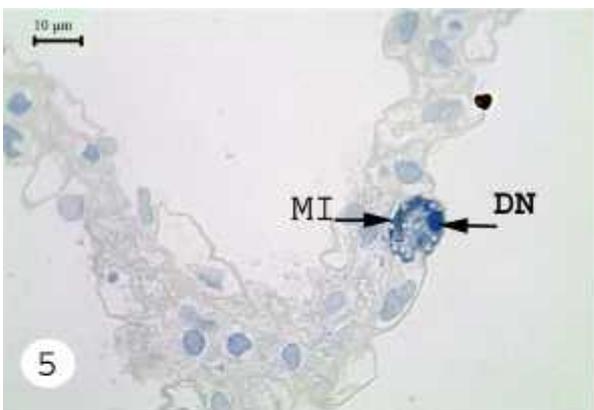
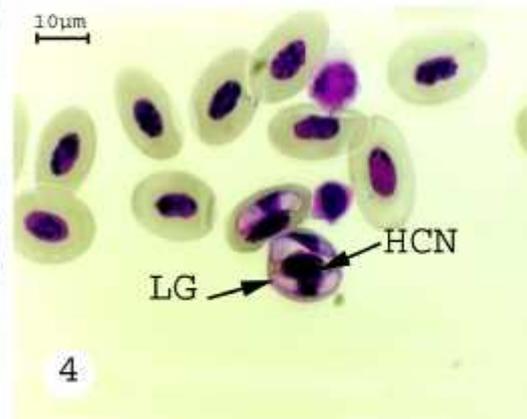
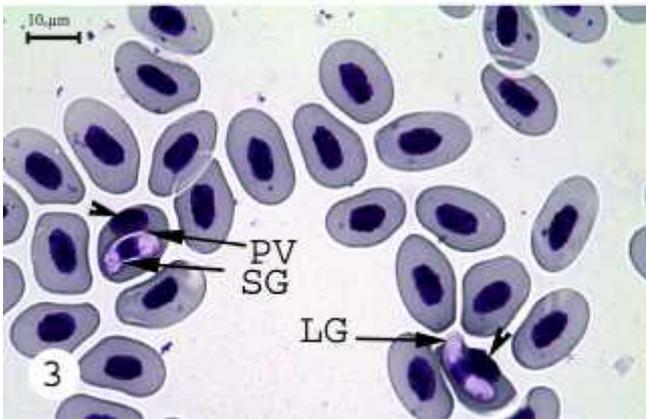
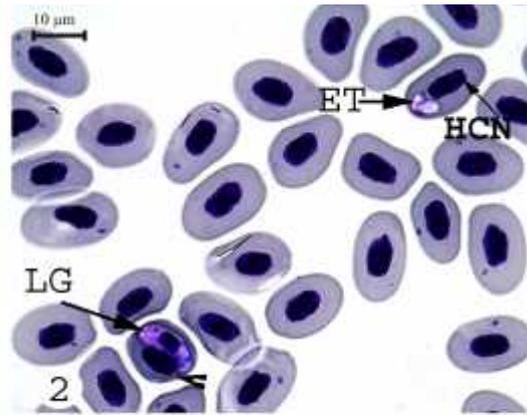
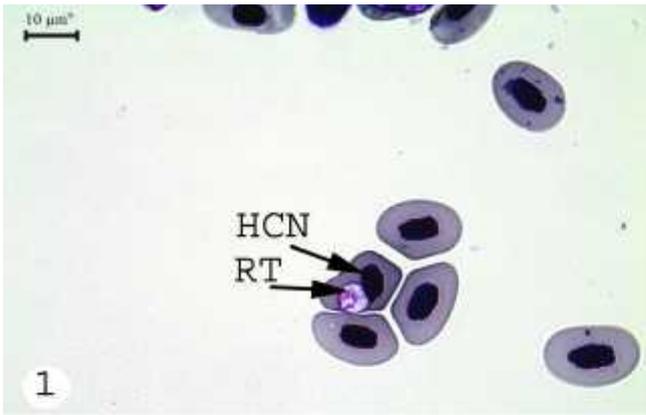
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List of Figures

Figs. 1-4: Light micrographs of Giemsa stained blood smears of *Haemogregarina* species naturally infecting gecko *Tarentola annularis*: Fig. 1: Rounded trophozoite (RT) with not affected host cell nucleus (HCN); Fig. 2 Elongated trophozoite (ET) with not affected host cell nucleus (HCN), also showing long gamont (LG) and slightly displaced host cell nucleus (HCN); Fig. 3: Short gamont (SG) surrounded by parasitophorus vacuole (PV), and displaced host cell nucleus (arrow head), also showing long gamont (LG) and displace host cell nucleus (arrow head); Fig. 4: Double infected erythrocyte by long gamont (LG) without affection on host cell or its nucleus (HCN).

Figs. 5-8: Light micrographs of semi-thin sections in lung of *T. annularis* infected with *Haemogregarina* species: Fig. 5: A micromeront (MI) containing one daughter nuclei (DN); Fig. 6: Micromeront (MI) possesses four daughter nuclei (DN); Fig. 7: A macromeront (MA) contains peripherally arranged developing merozoites (M), and show residuale body (RB); Fig. 8: A macromeront (MA) contains developing merozoites (M).

Fig. 9: Transmission electron micrograph of macromeront in lung of *T. annularis* infected with *Haemogregarina* species, showing a macromeront (MA) surrounded by parasitophorus vacuole (PV) in lung endothelial cells containing developing micromerozoites (M) and amylopectin granules (AG) and residual body (RB).



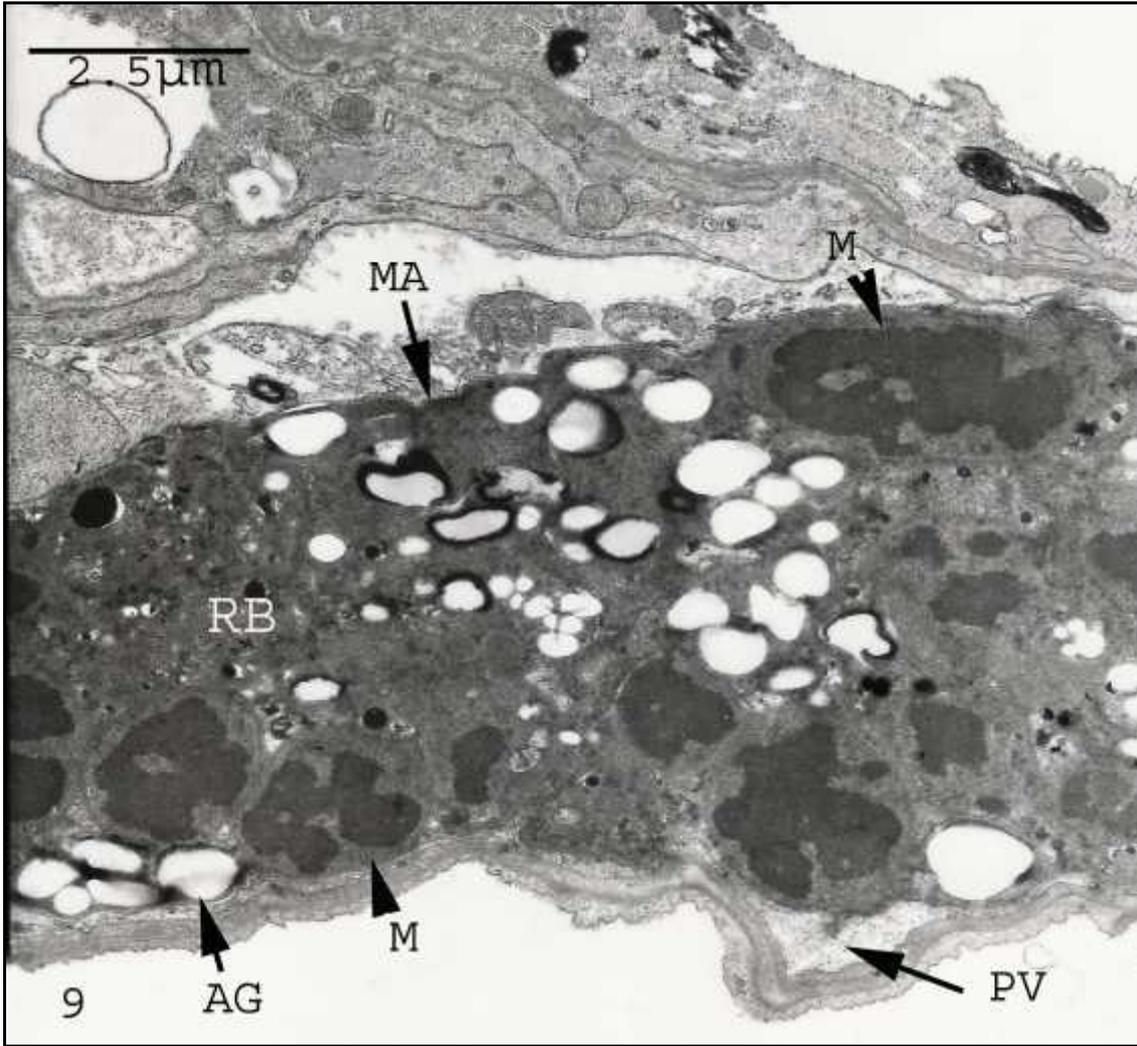


Table 1: Comparison between previously described haemogregarines species and that in the present study of Egyptian geckoes

Parasite	Host	Blood stages		Tissue stages		Locality	Reference
		Parasite stage	μm	Site	μm		
<i>Haemogregarina</i> sp.	<i>T. annularis</i>	Young thin form Young oval form Mature form	11.2 × 2.3 9.5 × 3.9 15.2 × 4.2	-	-	Asiut	El Naffar <i>et al.</i> (1991)
<i>H. tarentanulari</i> Mohammed, and Ramadan (1996)	<i>T. annularis</i>	SG LG	13-17 × 2.5-3.5	Lung and Liver	MISCH 28-34 × 13-17 MASCH 2-29 × 15-21	Abu Rawwash, Fsyoum, Aswan and St. Catherine	Saoud <i>et al.</i> (1995)
<i>H. rawwabi</i> Mohammed and Ramadan (1996)	<i>P. hasselquisti</i>	-	14-20 × 3.4-5	-	-	Abu Rawwash and 10 th of Ramadan	Saoud <i>et al.</i> (1995)
<i>H. helwanmohammedi</i> n. sp.	<i>Hem. flaviviridis</i>	-	17.5-27 × 3-4.5	Liver	Early schizont (16 × 10) Multinucleated schizont (22 × 13)	Abu Rawwash and 10 th of Ramadan Egypt	Saoud <i>et al.</i> (1995) Mohammed, and Ramadan (1996) (Cited from Saoud <i>et al.</i> , 1995)
<i>H. tarentannularis</i> Mohammed, and Ramadan (1996)	<i>T. annularis</i>	Young stage Growing stage Gametocyte	13-18 x5-8 12-19 x 4-5 13-17 x2.5-3.5	Lung and Liver	MASCH 28-34 x 19-25	Qena	Hussein (2006)
<i>Karyolytus</i> sp.	<i>P. hasselquisti</i>	SG LG	11.87 × 6.42 14.13 × 10.03	Lung and Liver	MI 14.93 × 13.14 MA 26.3 × 16	Qena	Rabie <i>et al.</i> (2006)
Haemogregarine species	<i>P. hasselquisti</i>	SG LG	8 × 4 13 × 4	Lung and Liver	MI 12 × 9 MA 20 × 13	Egypt	Abd-Al Aal <i>et al.</i> (2009)
Haemogregarine species	<i>T. annularis</i>	SG LG	12.2 × 2.1 15 × 12.1	-	-	Egypt	Abdel-Baki and Al-Quraishy (2012)
<i>Haemogregarina</i> Danilewsky (1885)	<i>A. bockiatus</i>	Small form Large form	8 × 3.5 12 × 5	Liver	-	Egypt	Present study
<i>Haemogregarina</i> sp.	<i>T. annularis</i>	R-trophozoite E-trophozoite SG LG	3.84 (diameter) 4.42 × 2.8 10.82 × 3.30 14.67 × 3.96	Lung	MI (13.25 × 12) MA (19.75 × 13.25)	Qena	

μm Measurement in microns, - Undetectable, *A. acanthodactylus*, **ET** Elongated trophozoite, *H. haemogregarina*, **Hem** Hemidactylus **LG** Long gamont, **M. Mahuya**, **MA** Macromeront, **MASCH** Macromerontschizont, **MI** Micromeront, **MISCH** Micromerontschizont, **P. Pyodactylus**, **RT** Rounded trophozoite, **SG** Short gamont, **T. Tarentola**