Light microscopic study of *Hepatozoon hamadaiensis* n. sp. (Apicomplexa: Hepatozooidae) from Elegant gecko, *Stenodactylus stenodactylus* (Gekkonidae) in Beharia Province, Egypt

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Abstract: Only one out of eighteen geckos was found infected with a *Hepatozoon hamadaiensis* n. sp. Erythrocytic stages were found intracellularly, however, free extracellular parasites were also seen. The latter measured about 13.3×2.4 µm. Intracellular parasites were only observed in the erythrocytes and none of the leucocytes were parasitized. The infected erythrocytes showed a slightly mechanical stretching and a considerable distortion. They measured 18.4×8.3 µm in an average, in comparing with 13.6×5.7 µm for non–infected ones. The host cell nucleus was displaced to the opposite side or pushed to one pole of the host cell. Erythrocytic stages were differentiated into three forms: i) **Small form**: It was oval, measuring 5.0–7.2×3.7–4.8 µm (L×W) and was considered as a merozoite that had recently penetrated the erythrocyte. ii) **Intermediate form**: It was elongate, measuring 6.7–8.1×4.4–5.6 µm (L×W). iii) **Large form**: It represented mature gamont. It was banana in shape, measuring 11.5–15.7×5.5–6.9 µm (L×W). Nucleus was rounded in shape, measuring about 2.9 µm in diameter. Merogonic stages were seen in the endothelial cells of lung capillaries. None merogonic stages were seen either in the circulating blood or skeletal muscles, brain, heart and kidneys. Uninucleate meront was elongate in shape, measuring about 8.6×6.7 µm. Early meronts having 4–6 nuclei and multinucleate ones were noticed, measuring 8.7×7.5 and 12.4×8.6 µm, respectively. A parasitophorous vacuole enclosed each developmental stage. Merozoites appeared as finger–like outgrowths on the meront surface. Two types of meronts were recognized: micro- and macromeronts. Micromeronts measuring about 16.3×14.0 µm, containing a less number (4–14) of macromerozoites that measured about 11.3×4.8 µm. Whereas, macromeronts measured about 30.4×20.2 µm, containing 20–34 micromerozoites, where each averaged 8.9×5.4 µm.

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1. Introduction

Haemogregarines are apicomplexans, in which gamonts infect blood cells of vertebrate hosts; merogony takes place in certain internal organs of these vertebrates, whilst both gamogony and sporogony occurs in invertebrate hosts (Levine, 1982). Genus *Hepatozoon* was first described in rats by Miller (1908) and then, it has been observed in all tetrapods as intermediate hosts and in numerous hematophagous invertebrates such as mosquitoes, mites, fleas, and ticks, which are their definitive hosts (Smith, 1996; Smith and Desser, 1997 and Telford, 2009). Species of *Hepatozoon* are among the most abundant and widely distributed hemoparasites. More than 300 *Hepatozoon* species have been identified (Allen *et al*., 2011), mostly based on occurrence of gamonts in the erythrocytes, or occasionally in the leucocytes of vertebrates (Herbert *et al*., 2010).

The present investigation describes both the erythrocytic and merogonic stages of *Hepatozoon hamadaiensis* n. sp. parasitizing the Elegant gecko, *Stenodactylus stenodactylus* by light microscopy.

2. Material and Methods

A total of eighteen geckos, *Stenodactylus stenodactylus* (Family: Gekkonidae) were handy captured from Kom-Hamada, Beharia Province. They were brought alive to Parasitology Laboratory, Zoology Dept., Fac. of Science, Al-Azhar Univ., identified according to Saleh (1997) and separately maintained. Geckos were microscopically examined for both blood and intestinal coccidian parasites as previously described (Abou El–Nour *et al*., 2012 and Abou El–Nour and El–Toukhy, 2014) as follows. 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 with light microscopy, small pieces of lungs, kidneys, liver, spleen, heart, gall bladder 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 the thin blood films were carefully examined microscopically and various developmental stages of the parasite were measured and photographed.

3. Results

Only one out of eighteen geckos was found a natural host of *Hepatozoon hamadaiensis* n. sp.

Erythrocytic stages

Erythrocytic stages of the parasite were found intracellularly (Figs. 1–6), however, few free extracellular parasites (Figs. 7&8) were also seen. The latter measured about 13.3×2.4 µm. Intracellular parasites were only observed in the erythrocytes and none of the leucocytes were parasitized. The parasites were enclosed within a parasitophorous vacuole. The infected erythrocytes showed a mechanical stretching and a considerable distortion, measured 18.4×8.3 µm in an average, in comparing with 13.6–5.7 µm for non–infected ones. The host cell nucleus was displaced to the opposite side or pushed to one pole of the host cell (Figs. 1–6), the parasites had no any karyolitic effect. Erythrocytic stages were differentiated into three forms: i) **Small forms:** They were oval, measured 5.0–7.2×3.7–4.8 µm (L×W) and considered merozoites that had recently penetrated the erythrocytes (Figs. 1&2). ii) **Intermediate forms:** They were elongate, measured 6.7–8.1×4.4–5.6 µm (L×W) and considered young gamonts (Fig. 3). iii) **Large forms:** They represented mature gamonts. They were banana in shape (Figs. 4–6), measuring 11.5–15.7×5.5–6.9 µm (L×W). Nucleus was rounded in shape, measuring about 2.9 µm in diameter.

Merogonic stages and merozoites

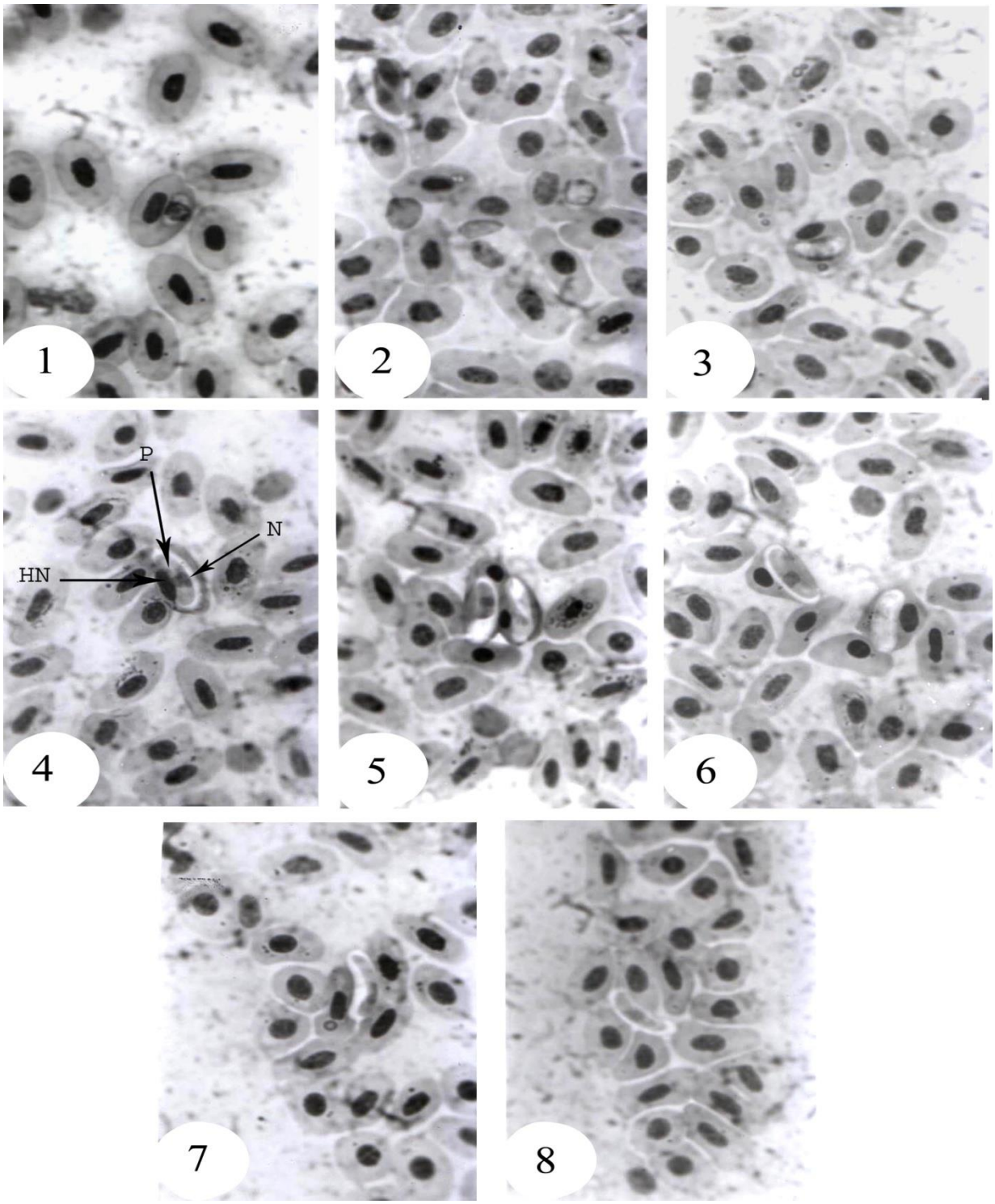
Merogonic process occurred in the endothelial cells of lung capillaries. None merogonic stages were detected in the circulating blood cells, skeletal muscles or any other organs. Upon leaving the erythrocytes, the parasite (sporozoite or later merozoite) invaded lung cells, became spherical and began to grow distinctly in size giving uninucleate meront, measuring about 8.6×6.7 µm were seen. Early meronts having 2–6 nuclei and multinucleate ones were observed, measuring 8.7×7.5 and 12.4×8.6 µm, respectively (Figs. 9–11). Meanwhile, a parasitophorous vacuole appeared enclosing the parasite and grew in size with the meront growth (Figs. 9–20). The meront nucleus was divided several times, and the resulting nuclei migrated to the periphery of meront underneath its outer border (Figs. 15–187), where merozoites usually developed (Figs. 18–20). Two types of meronts; micro- and macromeronts were observed. Micromeronts measured about 16.3×14.0 µm (Fig. 12) which gave rise 4–14 macromerozoites (Figs. 18&19) that measured about 11.3×4.8 µm. Whereas, macromeronts reached about 30.4×20.2 µm in size (Figs.13&14) and contained 20–34 micromerozoites, where each one measured about 8.9×5.4 µm (Fig. 20). The merozoites could enter new lung endothelial cells to develop into meronts or infect lung capillaries erythrocytes to grow into gamonts.

4. Discussion

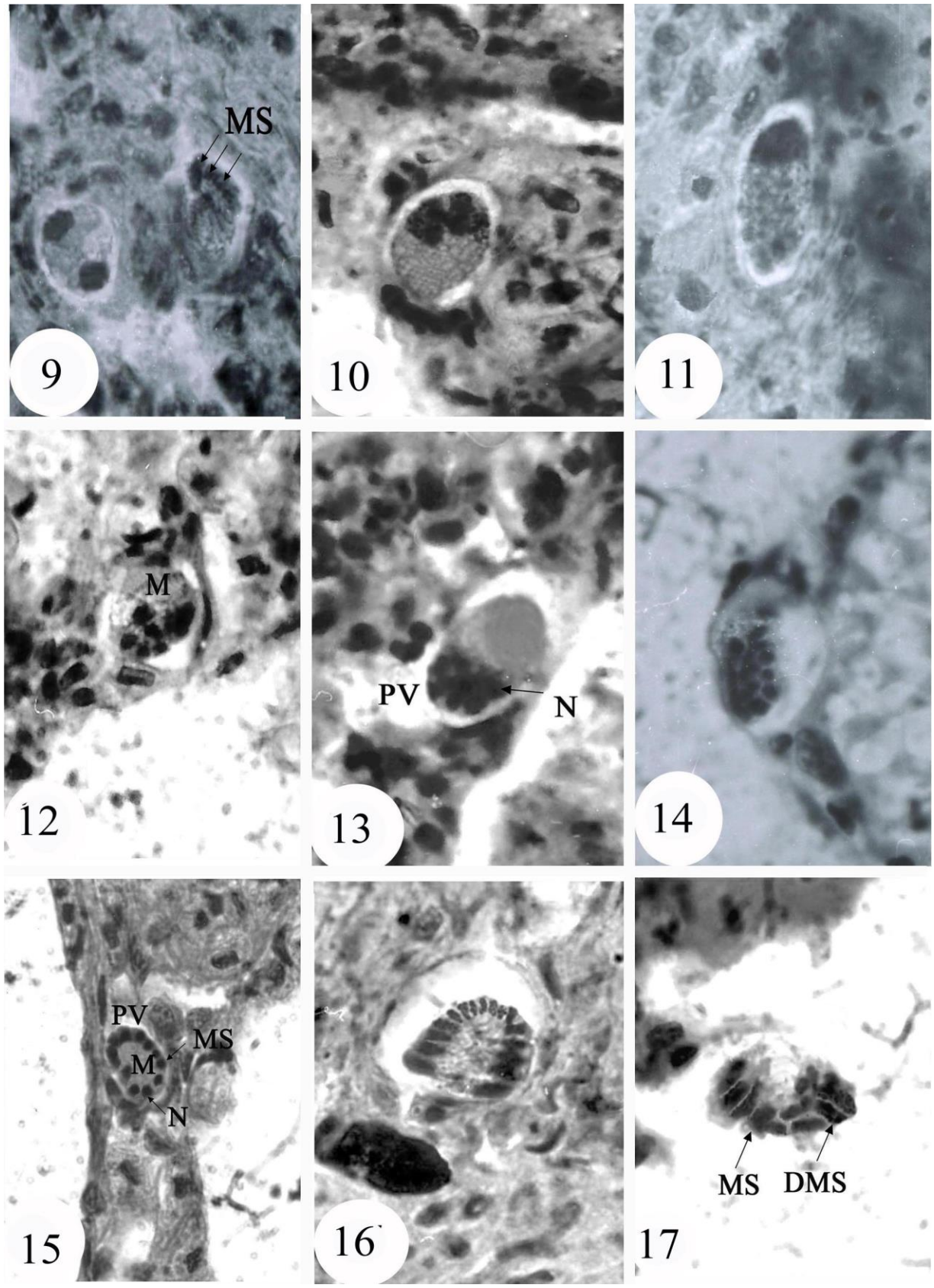
Generic identification of haemogregarines is based on some criteria such as characteristics of blood forms, merogonic stages, vertebrate and invertebrate hosts and characteristics of sporogonic cycle. The latter is an important criterion used to differentiate between the genera. But, the vectors and details of sporogonic cycle are unknown for the majority of haemogregarines. So, the designation of a haemogregarine to any genus is difficult.

However, 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*". Also, the systematic review of the haemogregarine complex which carried out by Smith (1996) has resulted in the expansion of genus *Hepatozoon* to include all members of genus *Haemogregarina* that infect all groups of tetrapod vertebrates. So, Smith transferred a total of 203 species of *Haemogregarina* (*sensu lato*) to the genus *Hepatozoon*. Therefore, some authors based their identification of haemogregarines after Siddall (1995) and Smith (1996), on only the developmental stages inside the vertebrate host (e.g. Abdel–Gawad *et al*., 2002; Shazly, 2003; Abou El–Nour, 2005; Abdel-Aziz *et al*., 2010; Yousef, 2015).

Considering the above mentioned discussion, the present parasite was placed under genus *Hepatozoon* along with many other haemogregarines infecting snakes and lizards. Whereas, it is also very important to study the vector and sporogonic cycle of such haemogregarines including the present parasite.



Figs. (1–8): Light micrographs of Giemsa-stained erythrocytic stages of *Hepatozoon hamadaiensis* n. sp. naturally infecting *Stenodactylus stenodactylus*: All photos x2200.
Figs. (1&2): Small forms. Fig. (3): Intermediate form. Figs. (4–6): Large forms, the host cell nucleus was forced to the opposite side of the parasite. Figs. (7&8): Extracellular parasites.
Nucleus (N), host nucleus (HN) and parasite (p).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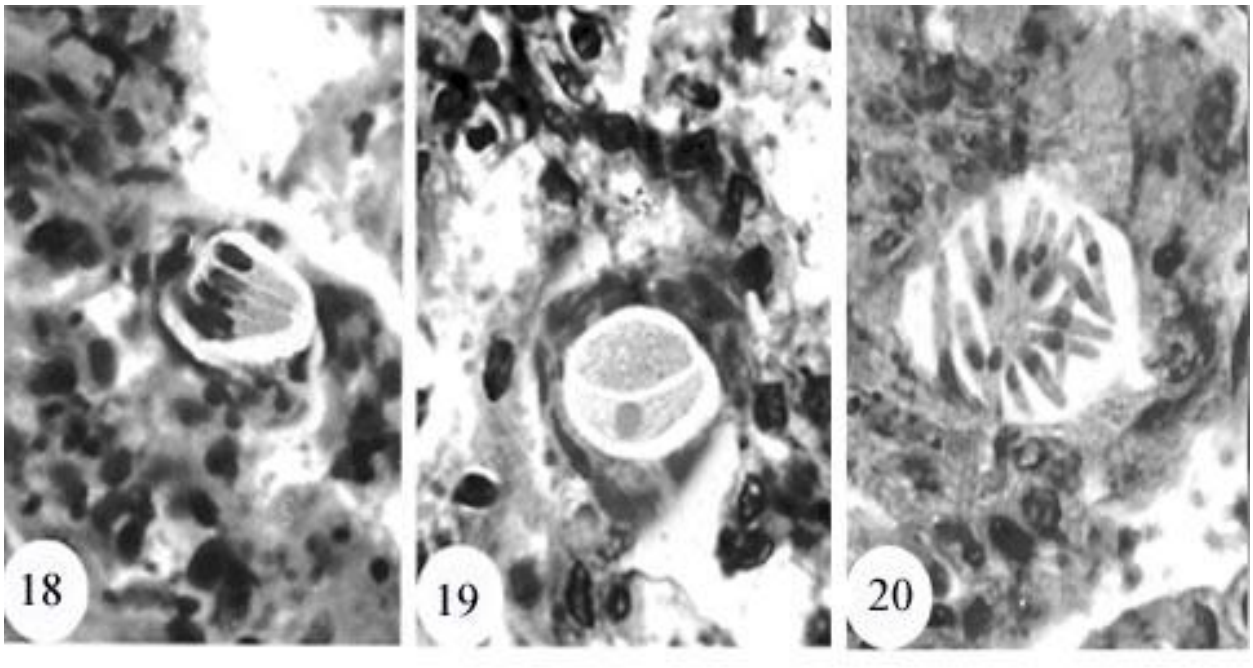

Figs. (9–20): Light micrographs of merogonic stages of the parasite in endothelial cells of lung capillaries (Haematoxylin–eosin stained sections). Each merogonic stage was enclosed by a parasitophorus vacuole. All photos x2400.
(Fig. 9): Early meront. Figs. (10&11): Multinucleate meronts. Fig. (12): Micromeronts with some daughter nuclei within a clear parasitophorous vacuole. Figs. (13&14): Macromeronts. Figs. (15–17): Beginning of the budding of developing merozoites as finger–like outgrowths from the outer border of macromeronts. Figs. (18 & 19): Micromeronts with fully formed macromerozoites. Fig. (20): Macromeront with micromerozoites still attached to the residual body. Merozoites (MS), meront (M), parasitophorus vacuole (PV), nucleus (N) and developing merozoites (DMS).

Also, species identification in haemogregarines has been rather unsatisfactory because of insufficient knowledge of their life histories (Mohiuddin *et al*., 1967). Identification of haemogregarines to the species level is based also on some criteria such as morphological characteristics and measurements of blood stages, effect of the parasite on both host cells and nuclei, the host and its geographical distribution. In the present study, blood stages invaded only the erythrocytes which showed a mechanical stretching and a considerable distortion in comparing with the non-infected ones. The host cell nucleus was not affected with the different forms of the parasite, however, it was displaced to the opposite side or pushed to one pole of the host cell. These results agreed with that obtained by several authors for other haemogregarines (e.g. Saoud *et al*., 1995 and Al-Farraj, 2008). Whereas, Hussein (2006) reported that the infected erythrocytes with a haemogregarine were hypertrophied with their nuclei either longitudinally stretched or split into two fragments.

In the current investigation, three forms of erythrocytic stages were noticed. Three forms of blood stages were also reported for other haemogregarines infecting geckkonid hosts (Table). Elwasila (1989) recorded only one form of gamonts in *Haemogregarina* sp. infecting *Tarentola annularis* in Sudan. Whereas, two forms of gamonts were detected in a *Haemogregarina* sp. prasitizing *Ptyodactyl*us *hasselquisti* in Egypt, in two haemogregarines infecting *P. hasselquisti* in Saudi Arabia, in a haemogregarine from *P. hasselquisti* in Egypt and in a *Haemogregarina* sp. infecting *Tarentola annularis* in Egypt (Abdel–Ghaffar *et al*., 1994; Ahmed *et al*., 1999; Hussien, 2006 and Rabie & Hussien, 2014), respectively. By comparing the measurements of gamonts of the other haemogregarines previously described from gekkonid hosts (Table 1) with that of the current parasite, some differences were noticed.

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

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)
			Lengh	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)
Haemogregarina tarentannulari	*Tarentola annularis*	3	13.0-17.0	2.5-3.5	5.0-6.0	2.5-3.5	Lung	22-29× 15-21	28-34× 13-17	27-35	16	Egypt	Saoud *et al.* (1995)
Haemogregarina rawashi	*Ptychodactylus hasselquisti*	3	14.0-20.0	3.5-5.0	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	16.0× 10.0	22.0× 13.0	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	22-28 × 15-20	28-35 × 25-31	37	16		
Two haemogregarines	*Ptyodactylus hasselquisti*	2	22.0	10.0	No data	No data	Lung	13.9× 10.1	20.9× 17.6	24	12	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	8-14	2-6	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	Abou El-Nour & El-Toukh, (2014)
Haemogregarina sp.	*Tarentola annularis*	2	14.1 - 16.5	3.3 - 5.5	No data	No data	Lung	13.25 × 12.0	19.75 × 13.25	No data	No data	Egypt	Rabie & Hussein (2014)
Hepatozoon hamadaiesis n. sp.	*Stenodactylus stenodactylus*	3	11.5-15.7	5.5-6.9	2.9	No data	Lung	16.3 × 14.0	30.4× 20.2	20-34	4-14	Egypt	The present study

Merogonic stages of the present *Hepatozoon* only occurred in lung of the infected geckos. Similar results for other haemogregarines were previously reported (e,g. Abdel–Ghaffar *et al*., 1994; Saoud *et al*., 1995; Ahmed *et al*., 1999; Abou El–Nour, 2005; Hussein, 2006; Abdel–Aziz *et al*., 2010 and Abou El–Nour and El–Toukhy, 2014). The presence of two distinct types of meronts in the current parasite, which were referred to as micro- and macromeronts on the basis of their size, was clearly in the line with the data obtained for many other haemogregarines (Table 1). However, some differences in the measurements of micro- and macromeronts and also in the number of merozoites in the both types of meronts of the current haemogregarine were observed when comparing with the data obtained from other authors for haemogregarines parasitizing the geckkonid hosts (Table 1). Furthermore, *Hepatozoon* under this investigation was the only haemogregarine found in the host genus *Stenodactylus* so far.

According to the above discusion including the comparison between the current parasite and the other haemogregarines previously reported from gekkonid hosts (Table 1), it seems to be justified to consider the

present parasite a new species and it is suggested to be named *Hepatozoon hamadaiensis*.

Taxonomic summary

Type host: The vertebrat host is *Stenodactylus stenodactylus*, the invertebrate definitive host is unknown.

Type locality: Kom Hamada, Beheira Province, Egypt.

Blood stages: Intraerythrocytic, three forms were recognized; small (5.0–7.2×3.7–4.8 µm), intermediate (6.7–8.1×4.4–5.6 µm) and large (11.5–15.7×5.5–6.9 µm).

Merogony: Occurred in the endothelial cells of lung capillaries. Two types of meronts were recognized; micromeronts measuring (16.3×14.0 µm) and containing 4–14 macromerozoites as well as macromeronts measuring 30.4×20.2 µm and containing 20–34 micromerozoites.

Etymology: The species name is derived from the name of the locality which the host was collected.

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