# HAEMOGREGARINES OF GECKOS IN EGYPT, TOGETHER WITH A DESCRIPTION OF HAEMOGREGARINA HELMYMOHAMMEDI N.SP.

#### BY

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# هيموجررينات الأبراص في مصر مع وصف نوع جديد: هيموجريجارينا حلميمحمدي

محمد فتحيع . سعود و نادية ف . رمضان شادية ح . محمد و سامية م . فوزي

سجل المؤلفون أوليات الدم الطفيلية المعروفة بالهيموجريجارينات من ثلاثة أنواع من الأبراص (زواحف برية صغيرة) تم جمعها من عدة أماكن في مصر . وقد تضمن البحث إجراء مراجعة تحليلية ومناقشة موسعة للأراء العلمية التي سبق أن أبداها العديد من الباحثين المهتمين بدراسة هذه المجموعة من الأوليات الطفيلية ، خلص بعدها المؤلفون إلى ترجيح الأخذبنهج علمي سبق اقتراحه منذ فترة واتبعه بعد ذلك عدد من الباحثين الثقاة في هذا الجال ، كما تضمن البحث وصفاً لثلاثة أنواع من تلك الطفيليات هي : هيموجريجارينا تارنتانيولاري ، هيموجريجارينا رواشي بالاضافة إلى هيموجريجارينا حلميمحمدى وهو نوع جديد يوصف لأول مرة . وقد ناقش المؤلفون الأسس التي عكن الاعتماد عليها في تمييز الأنواع الثلاثة عن غيرها من الأنواع التي سبق تسجيلها في تلك المجموعة من الزواحف البرية .

Key Words: Blood parasites, Haemogregarines, Geckos from Egypt.

#### **ABSTRACT**

Three species of geckos collected from various localities in Egypt were examined for protozoan blood parasites. Haemogregarines were reported in all the three species of geckos. Problems of the generic and specific identification of haemogregarines are reviewed and a practical policy suggested earlier is discussed, updated and followed in the present work. Haemogregarina tarentannulari Mohammed and Ramadan, 1996 and H.rawashi Mohammed and Ramadan, 1996 are redescribed from 44.4 and 43.8% of Tarentola annularis and Ptychodactylus hasselquisti respectively and their geographical distribution is extended to new localities in Egypt. H.helmymohammedi n.sp. is described from Hemidactylus flaviviridis collected from Southern Sinai. Various blood forms and some tissue stages are described for the three species of haemogregarines which are also compared with other related species from Africa.

#### INTRODUCTION

Several records of haemogregarines from geckos in various parts of Africa are known. Plimmer [1] reported haemogregarines in *Tarentola annularis* and *Ptyodactylus lobatus* from Egypt. The blood forms of *Haemogregarina platydactyli* Billet, 1900 were redescribed from *Tarentola annularis* in Algeria [2]. *Hepatozoon burneti* was reported from *Tarentola mauritanica* in Southern Tunisia [3]; that parasite had been described earlier from the same gecko in

Tunisia [4].

Riding [5] described a haemogregarine from *Tarentola annularis* in the Sudan. Saoud and Younis [6] gave a brief description of a haemogregarine from the same host in the same locality. El Wasila [7] found that *Tarentola annularis* in the area around Khartoum, Sudan had *Haemogregarina sp.* which was fully described.

Ramadan [8] made a camprehensive study on the protozoan blood parasites of reptiles in Egypt. Abdel Ghaffar et al. [9] described Haemogregarina sp. from the gecko, Ptychodactylus hasselquisti in Egypt.

Mohammed and Ramadan [10] in their study on haemogregaines of Egyptian geckos, described *Haemogregarina tarentannulari* from 74.3% of *Terentola annularis* collected from Cairo, Abu Rawash and Qenna, *H. rawashi* from 66.6% of *Ptychodactylus hasselquisti* from Giza and Abu Rawash and *H.sp.* from 4.6% of *Tarentola mauritanica* from Borg Al Arab and Ameriya near Alexandria.

The present study was undertaken to augment our knowledge on the blood parasites of Egyptian reptiles with particular reference to geckos. Special attention has been given to examine reptilian species from areas not previously covered in other studies.

#### MATERIAL AND METHODS

Geckos were collected from certain localities in Egypt, including Abu Rawash, Fayoum and Aswan in Upper Egypt, 10th of Ramadan, Ballah and Ismaiylia in Eastern Delta and St. Catherine in Southern Sinai. Animals were collected during the period 1987-1992, brought alive to the laboratory in Cairo and brief notes on the dates and sites of collection together with measurements of reptiles were recorded. Identification of geckos was based on "Checklist of the reptiles and amphibians of Egypt" [11].

Peripheral blood was obtained by snipping the tip of the tail. Blood was examined in air- dried, methanol-fixed blood smears stained in Giesma's stain[10]. Serial sections of lungs, liver and spleen together with bone marrow smears were examined from infected reptiles for exoerythrocytic stages of haemogregarines. These organs were dissected from infected animals and cut into small pieces. They were fixed either in Bouin's fluid for 24 hrs. or in Carnoy's fluid for  $2^{1}/_{2}$  hrs, dehydrated and mounted in paraplast. Serial sections, 5  $\mu$ m thick were stained in Delafield's haemotoxylin and counterstained in eosin, then dehydrated, cleared and mounted in D.P.X. in the usual way.

Drawings were made to scale, using camera lucida. Measurements were made from these drawings, using a calibrated divider. Photomicroscopy was made using Nikon AFX research photomicroscope.

#### RESULTS

# I. GENERAL PREVALENCE OF HAEMOGREGARINES IN GECKOS

A total of 225 geckos, belonging to three different species collected from certain localities in Egypt were examined for blood parasites (Table 1). Of these, 106 had pure haemogregarine infections; no other blood parasites

Table 1
Prevalence of Haemogregarines in Geckos
Examined from Certain Localities in Egypt

		No.	Positive for Haemogregarines		
Geckos	Localities	Examined	No.	%	
	Abu Rawash	59	37	62.7	
	Fayoum	77	31	40	
	Aswan	4	1	25	
Tarentola annularis	Ismailiya	11	2	18	
(White-spotted gecko)	El omaid	. 10	0	0	
	St Catherine	1	1	100	
	Total	162	72	44.4	
	Abu Rawash	48	25	52,1	
	Aswan	6	0	0	
Ptychodactylus hasselquisiti	10th Ramadan	15	7	46.6	
(Fan-footed gecko)	St. Catherine	4	0	0	
. 2 7 4	Total	73	32	43.8	
	Abu Rawash	10	0	0.	
Hemidactylus flaviviridis	10th Ramadan	6	0	0	
(Yellow-bellied house gecko)	St. Catherine	4	2	50	
	Total	20	2	10	

were observed during the present work.

# II. SPECIES OF HAEMOGREGARINES IN INFECTED GECKOS

# A. Haemogegarina tarentannulari Mohammed and Ramadan, 1996

Blood forms and tissue stages were reported in *Tarentola* annularis caught from Abu Rawash, Fayoum, Aswan and St. Catherine. Various blood forms were shown in red blood corpuscles while tissue forms were observed in both the lungs and liver of infected geckos.

#### 1-Blood Forms (Plate I and II)

#### 1.1 Immature Thin Forms (Figs.1-3, 17 and 18)

These are thin and somewhat elongated, with two rounded ends but occasionally one of their extremities may be more pointed than the other (Figs. 2 and 3). The thin forms measure 6  $\mu$ m long and 2  $\mu$ m broad. The cytoplasm is finely granular, and stains faint blue with Giemsa. The nucleus which is small, and not compact measures about 2  $\mu$ m long and 2.5  $\mu$ m broad. It is formed of fine chromatin granules.

#### 1.2 Immature Oval Young Forms (Figs. 4 and 20)

This form is oval with two rounded ends, measuring 5.5  $\mu$ m long and 4  $\mu$ m broad. The nucleus which occupies approximately half the length of the parasite is formed of darkly stained chromatin filaments. The nucleus measures

 $3 \mu m$  long and  $4 \mu m$  broad and the cytoplasm is vacuolar.

#### 1.3 Growing Trophozoites

These forms are of two types:

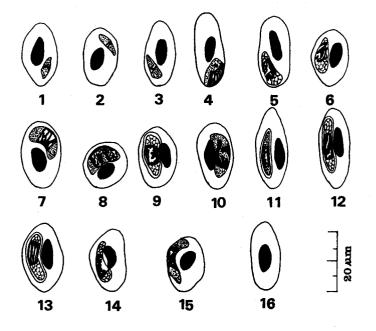
#### 1.3.1 Oval Trophozoites (Figs. 5-10 and 19)

They are broad, oval forms with two rounded ends, but one of them may be thinner than the other. They have a centrally situated nucleus which is compact and stains dark pink with Giemsa stain (Fig.19). The cytoplasm is somewhat vacuolar. They range in size between 13.2 and 16.4  $\mu$ m long and 5 and 8  $\mu$ m broad. The nucleus measures between 6 and 9  $\mu$ m long and 5 and 8  $\mu$ m broad. These oval trophozoites lie along the length of the erythrocytes. Neither the position of the host cell nucleus nor its shape are markedly changed. Also, these parasite stages have no effect on the size of the host cell.

### 1.3.2 Elongate Trophozoites (Figs.11-13, 21 and 22)

Giemsa-stained trophozoites appear as sausage shaped intra-erythrocytic bodies that lie parallel to the longitudinal axis of the erythrocytes. These forms have either similar rounded ends (Fig.13) or sometimes one end is narrower than the other (Figs. 11 and 12). The trophozoits are contained in a capsule, forming around them a parasitophorous vacuole. They range in size between 12 and 19  $\mu$ m long and between 4 and 5  $\mu$ m broad. They usually curve around

#### PLATE I



### PLATE I

Haemogregarina tarentannulari Camera-lucida drawings of blood forms from dry-fixed smears stained with Giemsa.

Figs. (1-3): Young thin form. Fig. (4): Oval young form.

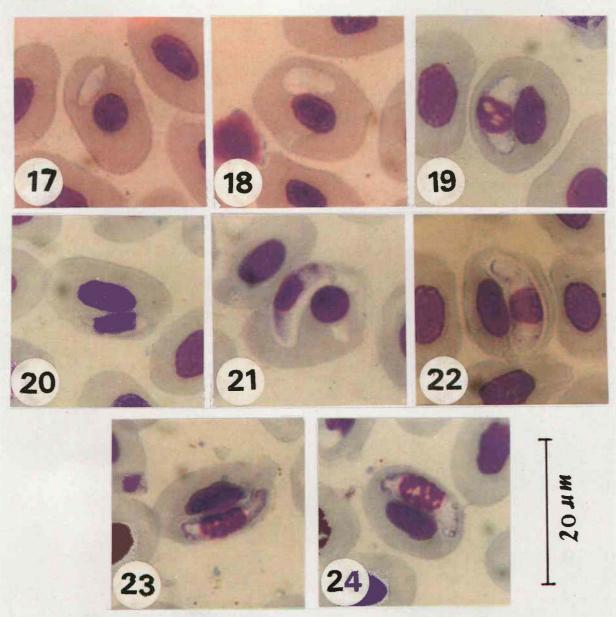
Figs. (5-9): Oval trophozoites.

Figs. (10-13): Encapsulated elongate trophozoites showing different shapes of nucleus.

Figs (14&15): Gametocytes.

Fig. (16): Normal, uninfected erythrocytes.

### PLATE II



### PLATE II

Haemogregarina tarentannulari Photomicrographs of blood froms from dry-fixed smears stained with Giemsa.

Figs (17&18): Young thin forms.
Fig. (19): Oval trophozoite, showing the structure of the nucleus.

Fig. (20): Oval thin form.
Figs. (21,22): Different forms of elongate trophozoites.
Figs. (23,24): Gametocytes.

the contour of the host cell, causing it to become stretched and frequently they push the host-cell nucleus away from its central position (Fig.11). The nucleus is centrally situated and occasionally is pushed towards one of the two ends (Fig.13); it is rectangular in shape and extends across the width of the parasites. The nucleus is formed of dark bands connected to each other with thin chromatin filaments (Fig. 13); it stains dark pink with Giemsa's stain. It ranges in size between 4 and 7.5  $\mu$ m long and between 3 and 5  $\mu$ m broad. The cytoplasm is vacuolar.

### 1.4 Gametocytes (Figs. 14, 15, 23 and 24)

These are slim elongate forms having either dissimilar rounded ends (Fig. 14) or one of them is narrower than the other (Fig. 15). They are curved around the nucleus and range in size between 13 and 17 $\mu$ m long and between 2.5 and 3.5  $\mu$ m broad. The nucleus is centrally situated (Fig.15) or sometimes pushed towards one end (Fig. 14). It is darkly stained and formed of dark chromatin clusters and measures between 5 and 6  $\mu$ m long and 2.5 and 3.5  $\mu$ m broad. The cytoplasm is faintly stained, having a vacuolar appearance with some dispersed red granules at the two ends.

#### 1.5 Effect of the Parasite on the Host Cell

The host erythrocytes undergo hypertrophy which is slight or marked, depending on the size of the parasite. Broad oval forms enlarge the breadth of the host cell, while elongate forms produce less effect on their host blood cells. Anisocytosis with displacement of the host-cell nucleus is observed although the host cell still retains its shape and size.

#### 2. Schizogony (Plate III, Figs. 26-35)

Asexual reproduction occurs only in the internal organs; no shizogonic stages were observed in the peripheral blood. Various developmental schizogonic stages were frequently seen in the lungs and liver.

The youngest schizogonic stage seen in the lung sections is oval, uninucleated and measures about 12.6 $\mu$ m long and 8  $\mu$ m broad. The vacuolar cytoplasm contains a rounded centrally situated nucleus, measuring about 6  $\mu$ m in diameter (Fig. 25). Binucleated schizonts (Fig. 26) are oval, measuring about 21  $\mu$ m long and 14.4  $\mu$ m broad; each of the two rounded nuclei measures about 2.4  $\mu$ m in diameter and are situated at opposite sides. As division proceeds, quadrinuclated schizonts (Fig. 27) are also detected in sections of the lungs; they are more or less oval, measuring 13.8  $\mu$ m long and 9  $\mu$ m broad with rounded nuclei, each measuring 3  $\mu$ m in diameter.

Multinucleated schizonts are of two types:

### 2.1. Macromeroschizonts

Successive nuclear divisions lead to the production of

multinucleate schizonts, which are either rounded (Fig. 28) measuring about 18  $\mu m$  in diameter or ovoid (Fig. 29), measuring 22 to 29  $\mu m$  long by 15 to 21  $\mu m$  broad. Macromeroschizonts produce about 16 merozoites, each of which is sausage-shaped (Figs. 30 and 31), measuring from 16.2 to 19  $\mu m$  long and from 4.8 to 6  $\mu m$  broad; their cytoplasm is vacuolar and their nuclei which measure 3  $\mu m$  in diameter are rounded and usually centrally situated.

#### 2.2 Micromeroschizonts

These are ovoid (Figs. 32 and 33) measuring from 28 to 34  $\mu$ m long and 13 to 17  $\mu$ m broad; a clear vacuole encloses the schizonts and enlarges during development. Micromersoschizonts contain 27-35 elongate nuclei, measuring about 4.8 x 1.8  $\mu$ m.

Micromerozoites (Figs. 34 and 35), which measure 7.2  $\mu$ m long and 2.4  $\mu$ m broad, are elongate with two similar rounded ends. They occupy most of the schizonts. They have granular, darkly stained cytoplasm and a centrally situated elongate nucleus.

# B. Haemogregarina rawashi Mohammed and Ramadan, 1996

Blood forms and certain tissue were reported in *Pty-chodactylus hasselquisiti* caught from Abu Rawash and 10th of Ramadan. Various blood forms were shown in red blood cells while certain tissue forms were observed in both the lungs and the liver, with more forms seen in the former than the latter. Since the features of the tissue stages of *H.rawashi* observed in the present work are essentially similar to those reported by Mohammed and Ramadan [10], the following redescription of that species will be only restricted to blood forms, where certain infraspecific differences are observed.

### Blood Forms (Plates IV and V) a. Immature Forms (Figs. 36-38 and 45)

These forms, which measure  $10-13.5 \mu m$  long and  $5-5.7 \mu m$  broad, are oval; both ends are rounded but occasionally one end is slightly narrower than the other (Fig.37).

The nucleus, which measures  $5.5 \times 5 \mu m$ , is centrally situated and formed of transversely extending chromatin bands. The cytoplasm is vacuolar and contains some red staining granules (Figs. 36, 37 and 45).

#### b. Growing Trophozoites (Figs. 39-42 and 46-49)

Trophozoites are broadly ovoid in shape with rounded ends but sometimes one end is much narrower than the other (Fig.41), and occasionally, it is bent to the inner side of the parasite (Figs. 40, 41 and 46). The trophozoites range in size between 20 and 25  $\mu$ m long and between 4.5 and 6  $\mu$ m broad. The cytoplasm is vacuolated and stains faint blue with Giemsa's stain. It has irregularly distributed red-

### PLATE III

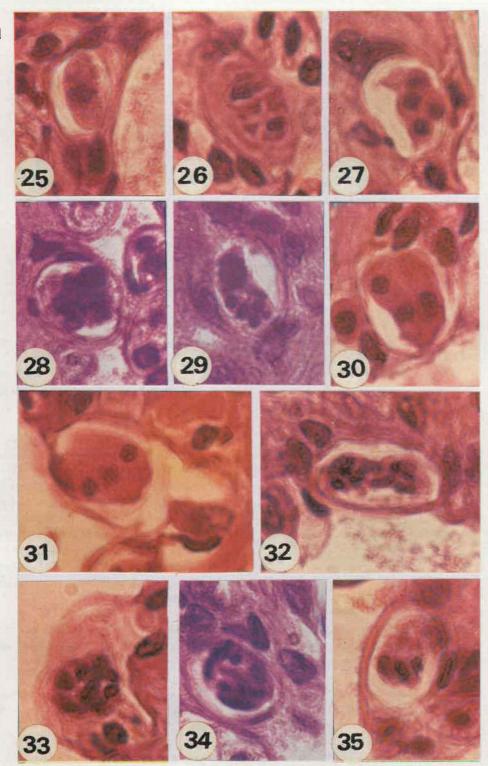


PLATE III

20 µm

Haemogregarina tarentannulari Photomicrographs of different schizogonic stages of the parasite from thin sections of the lungs stained with haematoxylin and eosin.

Fig. (25): Uninucleate elongate schizont. Fig. (26): Binucleate schizont. Fig. (27): Quadrinucleate schizont. Figs. (28,29): Macromerozoites. Figs. (32,33): Micromeroschizonts. Figs. (34,35): Micromerozoites.

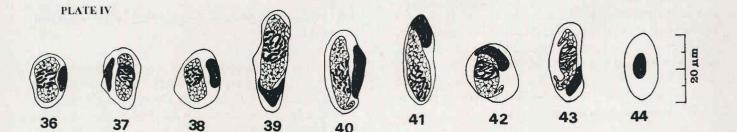


PLATE IV

Haemogregarina rawashi Camera lucida drawings of dry - fixed blood films stained with Giemsa.

Figs. (36-38): Young form showing darkly stained granules

Fig. (39): Trophozoite with the "cap" like host cell nucleus. Figs. (40-42): Trophozoites with narrower end which is bent to the inner side

Fig. (43): Gametocyte
Fig. (44): Normal uninfected erythrocyte



PLATE V Haemogregarina rawashi Photomicrographs of permanent preparations of dry-fixed Giemsa stained blood smears

Fig. (45): Young form Figs. (46-49): Trophozoites Figs. (50-51): Gametocytes

stained granules. The nucleus which comprises a network of chromatin filaments is rounded and occupies the broad part of the parasite; in rare cases, the nucleus is found at one end of the parasite (Fig. 41). The trophozoites normally occupy most of the host cells but are frequently seen lying adjacent to the host-cell nuclei. In some cases, the host-cell nucleus surrounds one end of the haemogregarine in the form of a cap (Fig.41). The nuclei range in size between 6-10 µm long and 5-6 µm broad.

#### c. Gametocytes (Figs. 43, 50 and 51)

Gametocytes are slender elongate forms with their two ends bent inward but one of them may be broader than the other (Fig. 51). They range in size between 14-20  $\mu$ m long and 3.5-5  $\mu$ m broad. The cytoplasm is granulated, having some red-staining granules. The nucleus is elongated and measures 8.3  $\mu$ m and 5  $\mu$ m broad. It is usually centrally situated and formed of transversely extending chromatin filaments.

#### d. Effect of the Parasite on the Host Cell

The presence of trophozoites causes distinctive changes in the appearance of the host-cells which may become hypertrophied. The infected cells become dehaemoglobinized, staining paler than uninfected ones. The host-cell nucleus which is normally displaced by the parasite, is elongate, compressed and becomes more intensely stained than the nucleus of uninfected erythrocytes. The nucleus becomes pushed to one side of the parasite, lying close to its surface, but sometimes becoming displaced to one end. Although in trophozoites the host-cell is hypertrophied, yet gametocytes do not cause a remarkable effect on the host cells.

#### C. HAEMOGREGARINA HELMYMOHAMMEDI N.SP.

Twenty Hemidactylus flaviviridis were examined for blood parasites; six were caught from the 10th Ramadan, ten from Abu Rawash while the last four were caught from St. Catherine in Southern Sinai. Two out of the four Sinai specimens, collected in August 1990, were infected with a large haemogregarine which was found to be different from all the known species of the genus Haemogregarina. This species is described below as Haemogregarina helmymohammedi n.sp. in the honor of the distinguished Egyptian protozoologist, Professor A.H. Helmy Mohammed, Department of Zoology University of Ain Shams, for his pioneering studies on the Protozoa in Egypt.

#### 1. Blood Forms (Plate VI and VII)

### 1.1 Immature Young Trophozoites (Figs. 52-55 and 68)

These forms, which measure 15-18.5  $\mu m$  long and 3-4.5  $\mu m$  broad, are elongate to oval with a nucleus occupying approximately the entire length except for the terminal part which is somewhat pointed (Figs. 52-55 and 68). The nucleus, which measures 11-14  $\mu m$  long and 3-4.5  $\mu m$  broad, is formed of transverse coiled chromatin bands. The cyto-

plasm is granular.

# 1.2 Growing and Mature Trophozoites (Figs. 56-60 and 69-71)

These are large sausage-shaped forms where one of the ends is oval while the other is either slightly tapered (Fig. 59) or sometimes curved (Figs. 57 and 58). Sometimes, both anterior and posterior ends are bent in opposite directions (Fig. 60). Fully formed trophozoites measure 16-25  $\mu m$  long and 3-5  $\mu m$  broad. The nucleus occupies approximately all the parasite leaving only a small area of cytoplasm at both ends. The clear cytoplasm occasionally contains a small number of reddish granules which are always found near one end of the parasite (Figs. 57 and 59). The large nucleus stains pink with Giemsa's stain and its chromatin comprises thick transverse strands. In fully formed individuals, the nucleus measures 13-22  $\mu m$  long and 3-5  $\mu m$  broad.

#### 1.3 Gametocytes (Figs. 61-66 and 72)

These are slim elongate forms, measuring 17.5-27  $\mu m$  long and 3-4.5  $\mu m$  broad, with one oval end while the other end being slightly swollen and bent. The gametocytes, which occupy approximately the whole length of the erythrocyte are usually curved towards the host-cell nucleus (Figs.61-66 and 72). In some cases, the host cell is stretched across its long axis and the parasite together with the host-cell nucleus are situated on the same axis (Fig. 64). The clear cytoplasm occasionally contains a few fine granules at either or both ends of the parasites (Figs. 62 and 66). The elongate nucleus, measuring 11-18  $\mu m$  long and 3-4.5  $\mu m$  broad, fills the entire parasite except a small amount of cytoplasm found at one or both ends of the parasites. The nucleus is formed of parallel strands of chromatin filaments staining more darkly than those of the trophozoite.

#### 1.4 Effect of the Parasite on the Host Cell

The host cell appears not to be greatly affected by the presence of immature stages of the parasite (Figs. 53 and 68), but both the trophozoite and gametocyte stages cause the erythrocytes to be hypertrophied, stretched and stain paler (becoming dehaemoglobinized) than in uninfected ones. The host-cell nucleus, which is usually displaced by the parasite, becomes elongated and compressed, staining more intensely than the nucleus of the uninfected erythrocytes. The nucleus is generally pushed towards one of the lateral sides of the parasite, lying close to its inner surface (Figs. 58,59,68,69 and 70), and in some cases, it becomes displaced to one of the two ends of the erythrocyte (Fig.64).

### 2. Schizogony (Plate VIII)

Only one kind of schizonts with uninucleate and multinucleated stages is observed in sections of liver in the two infected geckos. The youngest form is ovoid (Fig.73) with a rounded nucleus located almost in the middle of the vac-

PLATE VI





PLATE VI

Haemogregarina helmymohammedi Camera lucida drawings of dry-fixed blood smears stained with Giemsa.

Figs. (52-53): Immature young trophozoites

Figs. (54-55): Growing trophozoites. Figs. (56-60): Gametocytes Fig. (67): Normal uninfected erythrocyte















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PLATE VII

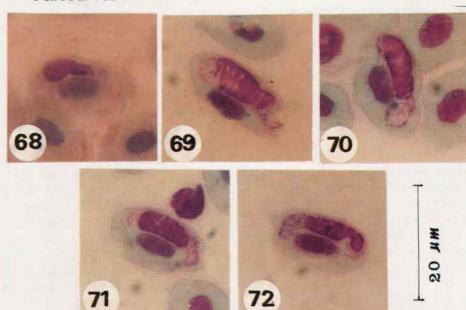


PLATE VII

Haemogregarina helmymohammedi Photomicrographs of preparations of dry-fixed Giemsa stained blood smears.

Fig. (68) Fig. (68): Young trophozoite Figs. (69-71): Growing trophozoites

Fig. (72) : Gametocyte

### **PLATE VIII**

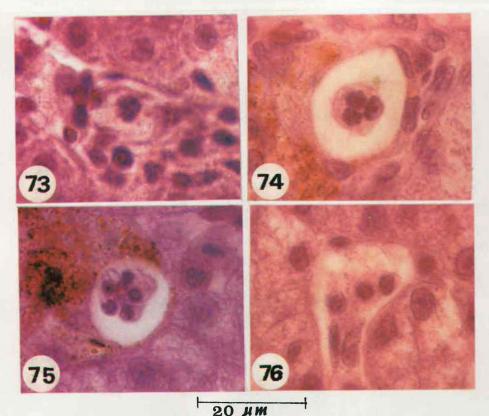


PLATE VIII

Haemogregarina helmymohammedi Photoicrographs of permanent preparations of thin liver sections, stained with haematoxylin & eosin.

Fig. (73): Uninucleate schizont

Fig. (74): A schizont

Figs. (75-76): Meroschizonts

uolar cytoplasm. This early schizont measures 16  $\mu m$  long and 10  $\mu m$  broad with the rounded nucleus, measuring 5  $\mu m$  in diameter. Stages possessing a few compact spherical nuclei (about 6) and surrounded by a clear parasitophorous vacuole, are observed (Figs. 74 and 75). A multinucleated schizont, measuring about 22 x 13  $\mu m$ , and comprising up to 21 elongate sausage-shaped merozoites with rounded nuclei are also observed in the liver sections.

#### DISCUSSION

The protozoan blood parasites detected in the present study are evidently haemogregarines. These are heteroxenous apicomplexan protozoa, in which gamonts infect red 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 [12].

### **Generic Identification**

Haemogregarines are included in the family Haemogregarinidae which contains the genera *Haemogregarina*, *Hepatozoon*, *Karyolysus* and *Cyrilia* [13]. Relating species of haemogregarines to one of these genera has posed certain practical problems [9,13] and several criteria have been suggested for the generic identification of haemogregarines, including the characteristics of blood forms, merogonic stages, types of vertebrate and invertebrate hosts and sporogonic stages [14]. It appears however, that these genera are best differentiated by the

characteristics of their sporogony [12,14].

However, the invertebrate vectors and details of the sporogonic stages are not known for the majority of haemogregarine species and accordingly, their designation to any of the known four genera becomes quite a problem. Mohammed and Mansour [14] suggested an alternative policy in which these blood parasites would be assigned to the genus Haemogregarina in its broad sense "senso lato". This usage, with proper indication could be a useful conservative approach [14,15,16,17]. Some authors followed this policy in practice while others preferred it as the best available alternative [18,19,20]. The same approach was concluded by Levine [12] who stated: "As a practical matter, unless they are piroplasmids, malaria parasites or related forms, most blood cells parasites of vertebrates are almost automatically called Haemogregarina. Later research may warrant shifts in the genus, but until such studies are carried out, the custom is warranted". Recently, the same practice was followed [9,10].

### **Specific Identification**

Specific identification of haemogregarines is a problem quite distinct in its nature from the question of generic determination [10,14,15,16,17]. After detailed discussion and careful consideration of the views presented by most authorities of the subject [14,15,,16,17,18.19.20.21.22], a practical and cautious policy for the specific identification of haemogregarines was suggested [8,14]. This included:

- 1-Following up blood forms over considerable period of time, whenever suitable material is available.
- 2. Accurate and detailed description of all blood forms and classifying them into stages. The description is augmented by adequate measurements and proper illustrations.
- 3-Careful observation of the various effects produced by blood forms on their host cells and their nuclei.
- 4-Careful examination of smears and sections of visceral organs for demonstration of the schizogonic stages.
- 5-Careful scanning of literature to compare the various characteristics of all the closely related forms of the previously recorded and described species with those of the species in question.
- 6-Specific identification is then made in the light of all the above information.

Species are regarded as new only when morphological and mensural data assure their distinctiveness from all other known species. Host and geographical distribution are also consulted to confirm the decision reached through morphological and mensural studies.

This approach was later followed in practice either fully or partly, by most of the recent investigators and texts dealing with haemogregarines [12,13,24]. Accordingly, this view has been adopted by the present writers as the best available practical policy.

#### Haemogregarines of Egyptian Geckos

In the present work, three species of haemogregarines are reported from Egyptian geckos: *Haemogregarina tarentannulari* Mohammed and Ramadan, 1996, *H. rawashi* Mohammed and Ramadan, 1996, and *H. helmymohammedi* n.sp.

Table [2] includes a comparison between *Haemogregarina* spp. of Egyptian geckos with other related species reported in Africa.

# 1-Haemogregarina tarentannulari Mohammed and Ramadan, 1996.

This species was described from 74.3% of *Terentola annularis* caught from Cairo, Abu Rawash and Qenna [8,10]. In the present work, the prevalence of this parasite in the same host species is lower (44.3%) and its geographical distribution is extended to other localities in Upper Egypt (Fayoum and Aswan), Lower Egypt (Ismailiya) as well as Southern Sinai (St. Catherine).

H.tarentannulari shows a certain resemblance with some stages of H.platydactyli Billet, 1900 as redescribed from Tarentola mauritanica in Algeria [2]. The mature forms of

the Algerian parasites have the same length as those of the Egyptian ones but they are distinctively broader, being 6-7  $\mu$ m compared to 2.5-3.5  $\mu$ m respectively. Immature forms of the Algerian parasites are much longer (13 x 3  $\mu$ m compared with 6.0 x 2.5-4  $\mu$ m). Some of the blood forms of the Algerian parasites have an attenuated end, a feature which is lacking in the Egyptian parasite. Foley and Catanei [2] described one type of schizonts which contained 1-8 elongate merozoites. This form of schizonts is distinctly different from micro-and macromeroschizonts described in *H.tarentannulari* from Egypt.

H. tarentannulari is differentiated from Hepatozoon burneti Lavier and Callot, 1938 described from Tarentola mauritanica in Tunisia by the characteristics of mature blood forms as well as the schizonts. As Mohammed and Ramadan [10] have appropriately indicated, the mature blood forms in Hepatozoon burneti, measuring 35 x 6 μm, are significantly larger when compared with those of H.tarentannulari. Moreover, schizonts in the former species are of one type and produce 10-20 much elongate and slender merozoites.

In *H.platydactyli*, it was noted that the only effect of the parasite on the infected erythrocyte was represented by displacement of the host cell nucleus but the cell was not hypertrophied [2]. In *Hepatozoon burneti*, the host cells became greatly deformed in the presence of very large parasites [3]. In *H. tarentannulari*, the host cells become hypertrophied and dehaemoglobinized, whilst the nucleus becomes displaced.

It appears that the characteristics of the haemogregarines reported previously in the same host from the Sudan [6,7] agree to a great extent with those of H. tarentannulari. This is particularly evident in the shape and measurements of thin and oval young forms as well as gametocytes [6]. However, El Wasila's description [7] included young and older meronts, measuring  $12 \times 14 \, \mu m$  with 1-6 merozoites and  $12 \times 16 \, \mu m$  with 16-25 nuclei respectively. Moreover, the gametocytes lay in a parasitophorous vacuole in the erythrocyte, a feature not described in the Sudanese and Egyptian and parasites [6,10].

# 2. Haemogregarina rawashi Mohammed and Ramadan, 1996.

This species was described from 66% of the fan-footed gecko *Ptychodactylus hasselquisti* collected from Abu Rawash and Giza in Egypt [10]. In the present work the parasite is recorded in 44.9% of geckos of the same species from Abu Rawash and its distribution is extended to the 10th Ramadan region in Eastern Delta.

Ramadan [8], in an unpublished comprehensive study on the protozoan blood parasites of some Egyptian reptiles, described for the first time haemoregarines from *Pty*chodactylus hasselquisti. In view of certain salient char-

aemogregarines of Geckos From Egypt

Table 2
Comparison Between Haemogregarines of Egyptian Geckos and Related Species in Africa

Parasites	Hosts and localities	Measurements and Remarks				
		Blood Forms			Effect on Host Cell	References
		Young and Growing forms	Gametocytes	Schizonts		
1-Haemogregarina sp.	Ptyodactylus lobatus, Egypt		short		Cells deformed	Plimmer (1912)
2- Н. sp.	Tarentala annularis, Egypt		short, bulky		Cells deformed	Plimmer (1912)
3- H. sp.	Tarentola mauritanica, Mediterranean		parasite foled over at one end, sometimes at both			Plimmer (1912)
4- H. platydactyli	Tarentola mauritanica, Algeria	13x3 µm elongate, enlarged at one extremity	14 - 16x6 - 7 µm, ellipsoidal elongate, sometimes pyriform, extremities rounded, one is attenuate.	12 x 17 - 8 x 36 μm in lungs & liver, ellipsoidal, from 1-8 elongate merozoites	not altered but the nucleus is only displaced.	Foley & Catanei (1925)
5- Hepatozoon burneti	Tarentola mauritanica, Tunisia	15x5 µm, banana- forms do not affect host cells	attain 35x6 µm, granules at extremities, no capsule, nucleus near one end.	abundant in lungs more than in the liver, each schizont contains 10 - 20 merozoites	greatly deformed, elongate, dehaemoglob. by the presence of big forms.	Lavier & Callot (1938)
6- H. sp.	Tarentola mauritanica, Egypt	young 10x.5 μm growing 13x5.6 μm			enlarged, dehaemoglobinized, host cell nucleus displaced	Mohammed & Ramadan, (1996)
7- H. tarentannulari	Tarentala annularis, Egypt	growing forms: oval: 14. 8x6.5µm elongate: 12-19 x 4-5 µm.	elongate and granular cytoplasm, 14 x 3 μm	schizogony in lungs & liver. Macromeroch. 23 - 29 x 15 - 21 \mu m Micromeroschizont 28 - 34 x 13 - 17 \mu m	hypertrophy of host cell, broad oval forms enlarge the breadth of the host cell, gametocytes produce the least effect.	Present work

## Table 2 (Cont.)

		Measurements and Remarks				References
Parasites	Hosts and localities	Blood Forms		Effect on Host Cell		
1 WE WOIT CO		Young and Growing forms	Gametocytes	Schizonts		
8 - H. sp.	Tarentala annularis, Sudan	thin: 8.1-9.2 x 2.2-3 μm oval: 6.9 x 2.2- 9.7 x 6.2 μm	12.4-15.9 x 3.2 - 2-5.5 μm		slightly hypertrophied, host cell nucleus displaced	Saoud & Younis (1969)
9- Н. sp.	Tarentola annularis, Sudan		gamonts: 12.5 x 3.6 µm, each gamont being situated in a separate parasitophorous vacuole	you meronts: 12x14 µm oval or irregular in shape with 1-5 merozoites, older meronts 12x16 µm with 16-25 peripherally arranged nuclei	erythrocytes do not show clear hypertrophy, infected endothelial cells highly hypertrophied	El-Wasila (1989)
10- H. tarentannulari	Tarentola annularis, Egypt	thin young 6x2.5 µm oval 6x4 µm growing oval 13-18 µm x5-8 µm, elongated 12- 19 µm x 4-5 µm	13-17 x 2.5-3.5 µm slim elongate, no capsule, cytoplasm has fine granules	schizogony in lungs and liver macromerosch. rounded or ovoid 28-34x19-25 µm, about 15 macromerozoites 30-40x15- 20 µm about 40 micro- merozoites	enlarged, dehaemoglobinized, nucleus displaced	Mohammed & Ramadan (1996)
11- H. rawashi	Ptychodactylus hasselquisti, Egypt.	thin: 7.5-10x3-4µm young oval 9.5- 16x4-8 um. Growing 22-31 x 5.5 - 9 µm. Broad with vacuolar cytoplasm	14-20x3.5 x 5 (17.5 x 4.5) μm, selender elongate with grangular cytoplasm	maromerosshizonts 22-28x15- 20 µm, 16 micromerozoites, micromeroschizonts, 28-35x25-31 µm about 37 micromerozoites	enlarged, dehaemoglobinized nucleus enlarged and displaced	Mohammed & Ramadan (1996)
12- H. rawashi	Ptychodactylus hasselquisti, Egypt	oval forms: 11.5 x 508 μm trophozoites: 20 - 25 x 4.5 - 6 μm	17.5-5 μm, elongate, cytoplasm granulated	macromeroschizonts 23x15 µm macromeroschizonts 29x25 µm oval or round 31 µm	trophozoites cause hypertroph, infected cells stain paler, nucleus is always displaced	Present Work
13- H. helmymohammedi n. sp.	Hemidactylus faviviridis. Egypt	young forms: 18 x 4.5 μm growing forms: 25 x 5 μm	27x4.5 µm slim elongate forms, curved towards the host cell nucleus & cytoplasm finely granulated	macromeroschizonts 22x13 µm in liver only, contain about 20 micromerozoites	trophozoites & gametocytes cause the erythrocytes to be stretched, nucleus is displaced	Present work

acteristics of both the blood and other tissue stages of these parasites which distinguished them from other species of the genus Haemogregarina, she suggested their designation to a new species. More than twenty years later, Abdel Ghaffar et al.[9] redescribed by both light and electron microscopy the blood and merogonic stages of what appeared to be the same species of parasites from the same host in Egypt. However, these authors, without discussing the diagnostic criteria used for characterizing this species assigned to the genus Haemogregarina senso lato, refrained from accepting Ramadan's suggestion to designate this haemogregarine to a new species. In their view, the 'identificaton' of a new species 'needs the examination of the final host of the parasite'. In the opinion of the present authors, the elucidation of the invertebrate host and knowledge of the details of sporogony are of paramount importance in validating the generic identification of haemogregarines in question but have nothing to do with specific identification of parasites belonging to this group of Apicomplexa. In this respect, reference is made to more than 150 species of haemogregarines described from both aquatic and terrestrial reptiles in the absence of any information about the identity of their invertebrate hosts or the characteristics of their sporogony [12]. Accordingly, the argument presented above by Abdel Ghaffar et al. cannot be accepted.

Mohammed and Ramadan [10] described the characteristics of blood forms (thin and oval forms, trophozoites and gametocytes) together with schizogonic forms (early schizogony, macromeroschizonts, micromeroschizonts, macromerozoites and micromerozoites). The present description of *H.rawashi* generally agrees with that of Mohammed and Ramadan [10], except for measurements of the macro-and micromeroschizonts. Although the number and measurements of the macro-and micromerozoites are similar in both descriptions, the measurements of the macro- and micromeroschizonts are slightly smaller than those given for this species [10].

H. rawashi was differentiated from H.tarentannulari by the larger thin oval young forms as well as the gametocytes, the larger trophozoites, the somewhat smaller macromeroschizonts, shorter macromerozoites [10].

The maturing blood forms of Haemogregarina platydactyli described from Algeria (2) shows certain similarities with the corresponding forms of H.rawashi. However, blood forms of the latter species are evidently larger (Table 2). Differences in schizogony are also evident between the two species. Both macro-and micromeroschizonts are known in H.rawashi but only one type of schizonts is known in H.platydactyli. Although the micromeroschizont of H.rawashi is similar in size with the schizont of H.platydactyli, the latter contains much fewer merozoites (1-8) compared with 37. Lastly, the effects of the parasites on the infected erythrocytes are different in the two species; the shape and size of infected erythrocytes are not affected in H.platydactyli, whereas hypertrophy and de-

haemoglobinization of those cells are observed in *H.rawashi*.

Haemogregarina rawashi is mainly differentiated from Hepatozoon burneti described from Tarentola mauritanica in Tunis [3] by the size and shape of gametocytes and their effect on infected blood cells and by the presence of two types of schizonts, and the number and size of the merozoites.

#### 3. Haemogregarina helmymohammedi n.sp.

In the present work, parasites of the genus *Haemogregarina* are described for the first time in geckos of the genus *Hemidactylus*. Only two infected geckos have been reported from one locality, but the characteristics of this haemogregarine, particularly its blood forms are strikingly different from other known species of the genus.

Although the infection in two geckos was low, many blood forms (maturing trophozoites and gametocytes) were clearly demonstrated. Only the liver was positive for the parasite schizonts. Two stages of trophozoites are observed in the blood: immature young and growing trophozoites in addition to gametocytes. The shape, and the characteristically large nucleus are distinctive features of this species of *Haemogregarina*. Schizonts were scarce in liver sections and although none were demonstrated in the lungs, the possibility of their presence in the latter organ cannot be excluded, in view of the limited number of geckos examined.

The shapes of immature young and growing forms of *H.helmymohammedi* being elongate or sausage-shaped-structures, having dissimilar ends, one being broader while the other is thin, are fairly similar to the corresponding stages described for *H.platydactyli* from *Tarentola mauritanica* in Algeria [2]. However, the much larger size of the nucleus clearly differentiates the former from the latter species. This feature also separates *H.helmymohammedi* from both *H.rawashi* Mohammed and Ramadan, 1996, *H.tarentannulari* Mohammed and Ramadan, 1996 and *Hepatozoon burneti* Lavier and Callot, 1938.

Gametocytes of *H.helmymohammedi* show certain resemblance to those described for *H.platydactyli* from Algeria [2] and *H.sp.* from the Sudan [7], being ellipsoidal or elongate with one round extremity while the other is attenuated or sometimes bent towards the inner side of the parasite. However, the gametocytes of *H.helmymohammedi* are much larger and thinner, measuring 17.5-27 x 3-4.5 µm compared with 14-16 x 6-7 µm and 12.5x3.6µm in Algerian and Sudanese material respectively. Moreover, the relatively larger nucleus of the gametocytes of the presently described species is different from the other two species.

Although certain features of schizogony in *H.helmymohammedi* (e.g. size and number of merozoites) clearly differentiate it from other related species of the ge-

nus, a full comparison has not been attempted in the present work in view of the limited material available. An examination of more heavily infected geckos may throw light on the characteristic features of schizogony in this species, especially as salient differences in the characteristics of the blood forms are usually reflected in the characteristics of tissue and other stages of these parasites [12,13,14].

The above mentioned differences together with the host and the restricted geographical distribution in Sinai clearly distinguish *H.helmymohammedi* as a new species.

#### **Specific Diagnosis**

Young trophozoites elongate oval with distinctively large nucleus; they measure 15-18.5  $\mu m$  x 3-4.5  $\mu m$  while the nuclei measure 11-14  $\mu m$  x 3-4.5  $\mu m$ . Mature trophozoites sausage-shaped, measuring 16-25  $\mu m$  x 3-3.5  $\mu m$  while the nuclei measure 11-22  $\mu m$  x 3-5  $\mu m$ . Gametocytes slim and elongate, measuring 17.5-27  $\mu m$  x 3-4.5  $\mu m$  while the nucleus measures 11-18  $\mu m$  x 3-4.5  $\mu m$ . Erythrocytes infected with mature trophozoites and gametocytes become hypertrophied and dehaemoglobinized. Meroschizonts in liver (?). They are of one type (?) and contain about 20 merozoites.

Host : Hemidactylus flaviviridis

Location: Growing trophozoites and gametocytes in

erythrocytes, schizogony in the liver (?).

Types : Syntypes deposited in the Protozoology Col-

lection, Department of Zoology, Faculty of

Science, University of Ain Shams.

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