

ON A NEW HAEMOGREGARINE OF *VARANUS GRISEUS* FROM EGYPT

By

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نوع جديد من الهيموجريجارينات في دم الورل الصحراوي من مصر

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يصف الباحثون نوعاً جديداً من الهيموجريجارينات في دم الورل الصحراوي من مصر ، ويشتمل البحث على وصف تفصيلي لمختلف مراحل الطفيلي التي تصيب خلايا الدم الحمراء ، وتؤدي إلى حدوث بعض التغيرات في تلك الخلايا وإزاحة أنويتها ، كما تم وصف مراحل نمو تلك الطفيليات في الرئتين والكبد ، فضلاً عن مقارنة النوع الجديد بالأنواع الأخرى التي سبق وصفها من زواحف الورل في إفريقيا .

Key Words: Haemogregarine roshdyi n. sp., Haemogregarine, *Varanus griseus*, Egypt

ABSTRACT

Ten monitors, *Varanus griseus* collected from various localities in Egypt were examined for protozoan blood parasites. A new species of the genus *Haemogregarina* sensu lato is reported from six of these monitors. Blood forms of *Haemogregarina roshdyi* n.sp. are described. These forms produce changes to the shape and structure of infected cells including stretching and slight dehaemoglobinization of infected cells and displacement of the nuclei. Despite of the low parasitaemia observed in the blood, schizogonic stages have been observed in the lungs and liver. The new species is compared with other species of haemogregarines described previously from varanids.

INTRODUCTION

Few species of haemogregarines have been reported from African varanids. Laveran[1] briefly described *Haemogregarina varani* from *Varanus niloticus* in South Africa. A year later, Nicolle and Comte[2] described *H. borreli* from *Varanus griseus* in Tunisia and indicated that *H. borreli* was clearly different from *H. varani* although no specific differences were given. Laveran and Pettit[3] gave a more detailed description of *H. varani* from *Varanus niloticus* in the Senegal. They also reported a haemogregarine from *Varanus griseus* in the same locality. Franca[4] reported *H. varani* from *Varanus niloticus* in Portuguese Guinea. Wolbach[5] described the life cycle of *H. toddii*

from *Varanus niloticus* in Guinea. Ball[6] reported *H. varani* with a concurrent infection of a little known karyolytic haemogregarine from *Varanus niloticus* in Kenya.

Although members of the genus *Varanus* are well known reptiles in Egypt they have not been examined in the two previous surveys published on the blood parasites of Egyptian reptiles[7,8]. In the present work, *Varanus griseus* was examined and a hitherto undescribed species of the genus *Haemogregarina* (sensu lato) reported. This species is named *H. roshdyi* n.sp. in the honour of the distinguished Egyptian zoologist, the late Professor M.A. Roshdy, D.Sc. (London), former Chairman, Department of Zoology, University of Ain Shams.

MATERIAL AND METHODS

Ten *Varanus griseus* were collected from both West and East Kantara, (Ismailia Governorate), the 10th of Ramadan City and from the Behaira Governorate in Egypt. Peripheral blood was obtained by snipping of the tip of the tail. Air dried methanol-fixed, blood smears were stained in Giemsa's stain. Serial sections of the lungs and liver were examined from infected monitors for studying the exoerythrocytic stages of haemogregarines. Small pieces of lung and liver were fixed in Carnoy's fluid for 30 minutes, dehydrated and mounted in paraplast. Serial sections, 5 μm thick were stained in Delafield's haematoxylin, counterstained in eosin and dehydrated, cleared and mounted in D.P.X. Drawings and measurements were made using a camera lucida and a calibrated divider respectively. Photomicroscopy was made using Nikon AFX microscope.

RESULTS

A - PREVALENCE

Six out of ten monitors examined during 1990 (a prevalence of infection of 60%) were found-infected with haemogregarines. All four monitors examined from West Kantara during May and September together with two of three specimens examined during October from East Kantara were positive for haemogregarines. However, the two specimens collected during November from the 10th of Ramadan City together with a single specimen collected from Behaira during July were uninfected.

B - DESCRIPTION

1- Blood forms (Plates I, II)

1-1 Young Trophozoites (Figs. 1-5)

The youngest forms observed are sausage-shaped with two rounded extremities, but occasionally one of them was slightly narrower than the other (Fig. 3). They measure 8 - 13 μm by 2 - 3.5 μm , with a centrally located nucleus measuring 3 - 7 μm by 2 - 3 μm .

1-2 Growing Trophozoites (Figs. 6 - 10 and 17 - 22)

These are sausage-shaped with two rounded extremities; they measure 10 - 18.5 μm by 3.5 - 5.6 μm broad. The nucleus is either situated centrally (Figs. 6, 7, 10, 17, 19 and 21) or located nearer one of either extremity (Figs. 8, 9 and 18). The nucleus measuring 4.5 - 10 μm x 3.5 - 5 μm , is formed of closely packed transversely extending chromatin filaments in which darkly stained spots are occasionally observed. The granular cytoplasm contains some scattered red-stained granules which are either fine and numerous (Figs. 8, 9) or coarse and less numerous (Figs 7, 10). A dense cluster of extranuclear chromatoid dots are sometimes located near the extremity of the trophozoite (Fig. 22). Young and growing trophozoites frequently occupy the central part of infected erythrocytes, pushing the host cell nucleus aside

(Figs. 6, 7, 10) but the trophozoites do at times occupy a marginal position (Fig. 1).

1-3 Gametocytes (Figs. 11-15 and 23-26)

These are crescent-shaped, with fairly swollen and inwardly curved ends, encircling the host cell nucleus (Figs 12-15); they measure 13 - 20 μm x 1.5 - 2.5 μm . Blue - stained or pinkish granules are frequently observed at either extremity (Figs. 24-26). The nucleus, measuring 6.0 - 8.5 μm by 1.5 - 2.5 μm occupies the entire width of the parasite and approximately half of its length. The finely granular cytoplasm is stained dark blue with Giemsa's stain.

1-4 Effect of the Parasite on the Host Cell

Infected erythrocytes show certain changes, especially in their shape, even when the parasites are small. Displacement of the host cell nucleus was common in all erythrocytic stages of the parasite. In most cases, the blood cells parasitized by elongate forms are longitudinally stretched and the host cell is slightly dehaemoglobinated.

2- Schizogony (Plate III)

Schizogony occurs in the lungs and liver but schizogonic stages are more common in sections of the lungs. The youngest developing schizogonic stage in the lung (Fig. 27) is round, uninucleated and measures 9 μm in diameter. The vacuolar cytoplasm contains an oval nucleus which measures 3.6 μm in diameter and is situated eccentrically in the early schizogonic stage. Multinucleated schizonts are of two types, namely macromeroschizonts and micromeroschizonts.

2-1 Macromeroschizonts

These schizonts are rare in sectioned material. They are either rounded (Fig. 28), measuring about 25 μm in diameter or oval (Fig. 29) measuring 20 - 28.4 μm x 12 - 19 μm . The largest schizonts contain about 20 nuclei which are round and measure 2.4 to 3.6 μm in diameter. The macromerozoites (Fig. 30) are elongate with one end broader than the other, and possess vacuolar cytoplasm and centrally situated round nuclei measuring 9.4 - 11.4 μm x 2 - 3 μm . The macromeroschizonts are usually surrounded by large parasitophorous vacuoles (Fig. 30) and contain no residual cytoplasm.

2-2 Micromeroschizonts

These are more frequent in tissue sections than the macromeroschizonts. They are oval in shape (Figs 31 and 32) measuring 27 - 36 μm x 14.4 - 15.0 μm but they are occasionally round (Fig. 33), measuring 26 μm in diameter. A maximum of 32 small elongate micromerozoites are located around a residual body inside each fully formed schizont. Micromerozoites are elongate with one end less rounded than the other; they measure 5.4 μm x 2.4 μm and possess central compact nuclei.

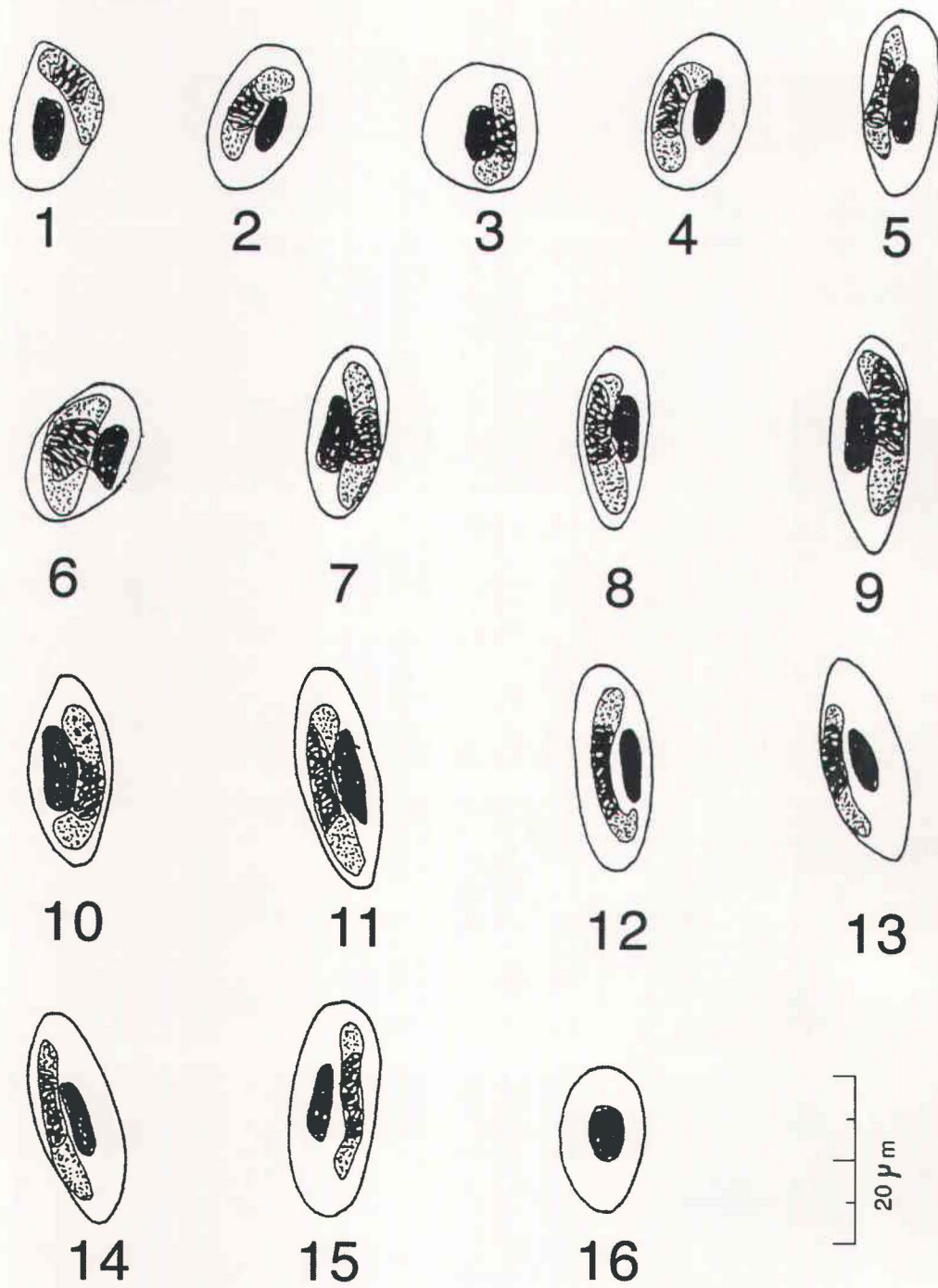
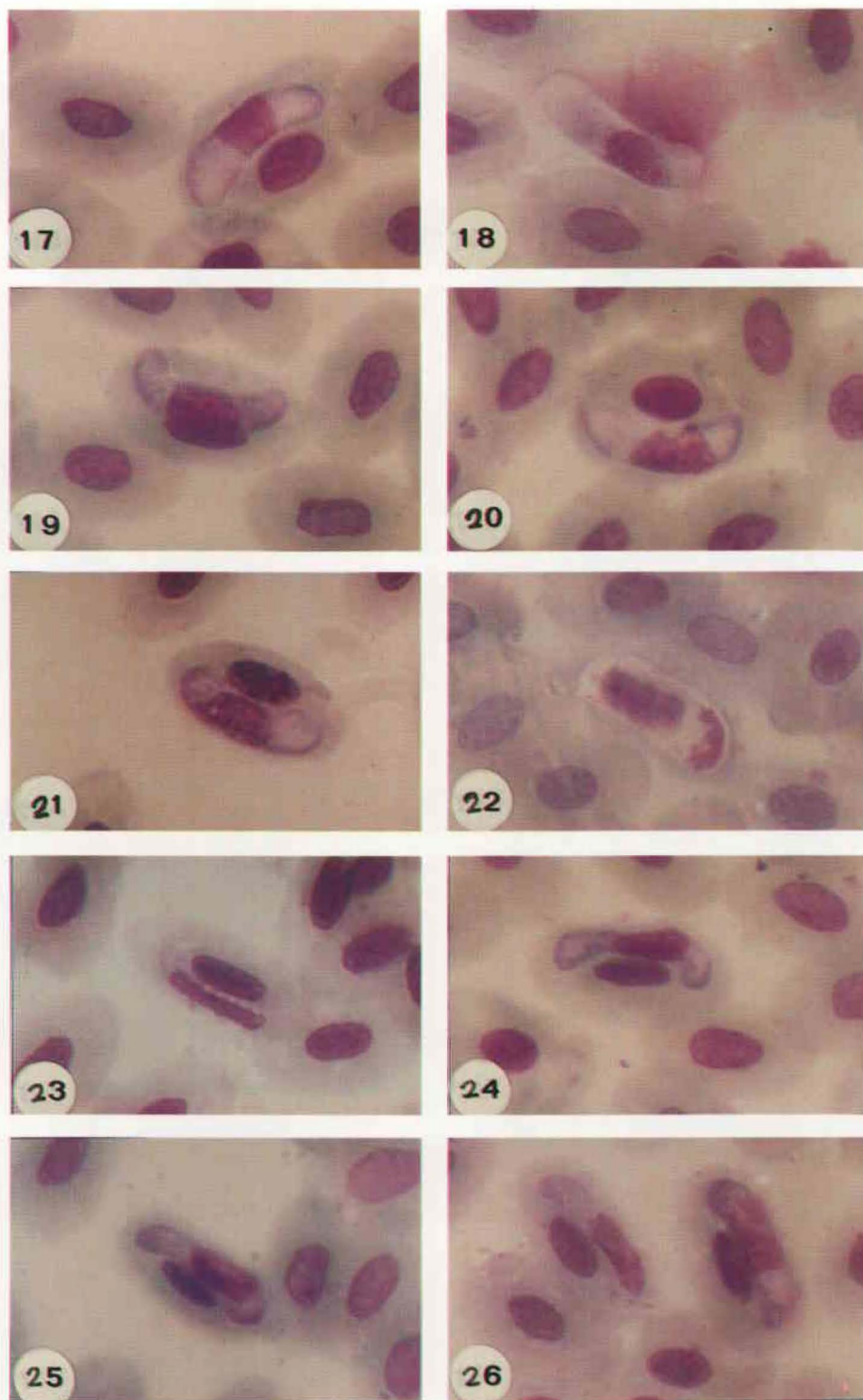


PLATE I

Haemogregarina roshdyi

The different blood forms from Giemsa's stained dry-fixed blood smears (Camera lucida drawings).

- Figs. 1-5 : Young trophozoites
- Figs. 6-10 : Growing trophozoites
- Figs. 11-15 : Gametocytes
- Fig. 16 : Normal uninfected blood corpuscle



20 μ m

PLATE II

Haemogregarina roshdyi

Photomicrographs of permanent preparations taken from dry-fixed blood smears

Figs. 17-22 : Different forms of trophozoites

Figs. 23-26 : Gametocytes

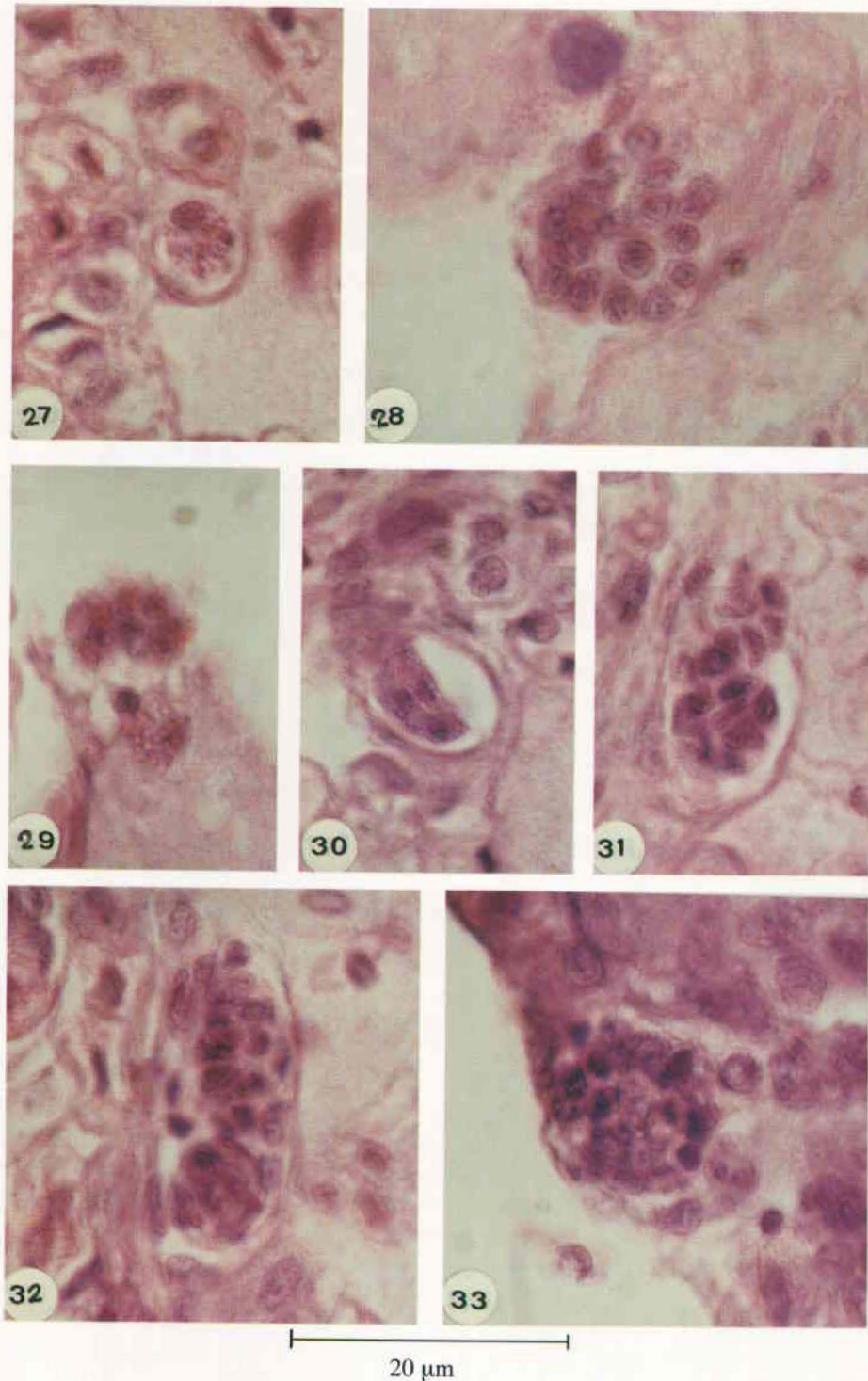


PLATE III

Haemogregarina roshdyi

Photomicrographs of permanent preparations of lung sections stained with haematoxylin and eosin

- Figs. 27 : Uninucleated macromeroschizont
- Figs. 28, 29 : Macromeroschizont
- Fig. 30 : Macromerozoites
- Figs. 31-33 : Mature micromeroschizonts

DISCUSSION

Haemogregarines are heteroxenous apicomplexan protozoans in which gamonts infect red blood cells of reptiles and other vertebrates. Merogony takes place in certain internal organs of vertebrate hosts whilst sporogony occurs in invertebrate hosts[8]. These blood parasites are included in the family Haemogregarinidae which contains four genera: *Haemogregarina*, *Hepatozoon*, *Karyolysus* and *Cyrlia*[9], but these genera are best differentiated on the basis of details of sporogony in their invertebrate hosts[10]. As the invertebrate hosts are not known for the majority of haemogregarines, it has been suggested that these blood gregarines should be assigned to the genus *Haemogregarina* in its broad sense (*sensu lato*)[8,11]. This approach has been adopted in practice[8,9,10,12,13,14] as the best available alternative in the absence of details of sporogony in the invertebrate host. Accordingly, blood protozoans described in the present work are included in the genus *Haemogregarina* (*sensu lato*).

In the present study, haemogregarines are reported for the first time from *Varanus griseus* in Egypt. Only 10 animals were examined for blood parasites, six of which were found to be infected with haemogregarines. Despite of the relatively low parasitaemia observed in the blood, schizogonic stages were detected in serial sections of the lungs and liver although these stages were much more frequent in tissues of the lung.

Three species of the genus *Haemogregarina* have been previously described from African monitors, namely *H. varani*, *H. borreli* and *H. toddi*[1-6]. However, Ball[6] suggested that *H. toddi* was probably a variant of *H. varani*.

Haemogregarina varani was originally described by Laveran[1] in South Africa and this was augmented later by a more detailed description of the same species in Senegal[3]. In the latter account, three different forms were described in the host's erythrocytes: small, medium and large forms, measuring 6 - 8 x 3 μm , 8 - 11 x 3 μm and 11 - 15 x 3 μm respectively. The largest blood forms were encapsulated, one of its ends being broadly rounded whilst the other end was pointed and folded on the body, giving the parasite a hook-like appearance. Schizonts measured 18 - 21 x 15 - 16 μm and two figured schizonts showed 4 and 32 macro- and micromerozoites respectively. Apparently, the small and medium forms in Laveran and Pettit's descriptions of *H. varani* represented the trophozoites, while the large forms were gametocytes.

Trophozoites of *H. roshdyi* n.sp. are obviously larger and have relatively larger nuclei than those of *H. varani*. Moreover, the shape and size of the gametocytes are different in the two species. In *H. roshdyi*, the gametocytes are relatively thin, encapsulated, sausage-shaped and possess swollen slightly curved ends; they measure 13 - 20 x 1.5 - 2.5 μm . In *H. varani*, one end of the encapsulated large forms (gametocytes) is broadly rounded while the other end was pointed and inwardly folded; they measure 11 x 3 μm . The number of macromerozoites in each schizont is also

different in the two species, being 20 and 4 in *H. roshdyi* and *H. varani* respectively.

Apart from the present record of haemogregarines in *Varanus griseus* in Egypt, two other records from the same host species exist, namely those of Nicolle and Comte[2] in Tunisia and Laveran and Pettit[3] in Senegal. In the former report, *H. borreli* was described from *Varanus griseus* while in the latter the same species of haemogregarines was recorded but without any meaningful description. In Nicolle and Comte's description, *H. borreli* was discovered in four out of five *Varanus griseus* examined in Southern Tunisia. One of the infected monitors showed an exceptionally high parasitaemia. The youngest erythrocytic forms had agranular cytoplasm and measured 3 μm in diameter. Other young forms (apparently trophozoites) measured 4 - 4.5 x 2 - 5 μm . Although the majority of infected erythrocytes showed little alteration, some of them were hypertrophic with pale cytoplasm and more or less karyolysed nuclei. The borders of the infected cells looked irregular and wavy and the parasites had abnormal protoplasm with poorly staining nuclei, indicating that both the parasites and the red cells were degenerating. Nicolle and Comte also produced figures, without description of larger encapsulated parasites, each with a pointed extremity, lying close to the erythrocyte's nucleus, while the other end was much broader. No schizonts were described.

Mackerras[15] suggested that Nicolle and Comte were dealing with a mixed infection of *H. varani* together with a karyolytic species, for which the name *H. borreli* should be valid. Ball[6] quoted Mackerras's suggestion without giving a definite opinion. However, the present authors are inclined to accept that suggestion in view of the close examination of Nicolle and Comte's figures in which encapsulated parasites similar to the large forms of Laveran and Pettit's *H. varani* are shown, in addition to the encapsulated karyolytic stages of the other species, *H. borreli*.

Although the blood forms of *H. roshdyi* n.sp. produce changes in the shape and structure of the infected erythrocytes, including displacement of their nuclei; there are no cases of disintegration or karyolytic nuclei similar to those reported in *H. borreli*. Therefore, in the absence of any description of the schizogonic stages in *H. borreli*, this karyolytic effect of the parasite on the host cell's nucleus clearly distinguishes it from *H. roshdyi* n.sp.

In conclusion, it is suggested that the above differences are sufficient to justify the identification of *Haemogregarina roshdyi* as a new species.

Specific diagnosis of *Haemogregarina roshdyi* n.sp.

Trophozoites and gametocytes occur in the host's erythrocytes. Trophozoites sausage-shaped, measuring 10.0 - 18.5 μm x 3.5 - 6.0 μm ; the nucleus measures 4.5 - 10 μm x 3.5 - 5.0 μm . Gametocytes crescent-shaped and measure 13 - 20 μm x 1.5 - 2.5 μm ; the nucleus measures 6 - 8.5 μm x 1.5 - 2.5 μm . Displacement of the host cell nucleus is common in all erythrocytic stages; the cell is longitudinally stretched and slightly dehaemoglobinized,

but no karyolysis of host cell nucleus is observed. Two types of schizonts are frequently seen in the lungs and less so in the liver. Macromeroshizonts are either rounded measuring 25 µm in diameter or oval measuring 20 - 28 µm x 12 - 19 µm. Each macromerozoite, up to 20 per schizont measures 9.4 - 11.4 x 2.4 - 3 µm. Micromeroshizonts are oval measuring 27 - 36 µm x 14 - 15 µm or rounded, measuring 26 µm in diameter. Each micromerozoite, up to 32 per schizont, measures 5.4 µm x 2.4 µm.

Host : *Varanus griseus*

Locality : West and East Kantara (Ismailia Governorate), Egypt

Types : Syntypes deposited in the Protozoological Collection, Department of Zoology, University of Ain Shams, Cairo, Egypt.

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Received 23 May, 1996