Journal of Bacteriology and Parasitology

El Maleck et al., J Bacteriol Parasitol 2014, 6:5
DOI: 10.4172/2155-9597.1000244

Research Article Open Access

Morphological and Ultrastructural of a New Species from Cephaline Gregarinidae Infected Fruit Egyptian Bat (*Rousettus aegyptiacus*) and its Vector

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Abstract

Out of 179 fruit bats *Rousettus aegyptiacus* examined, only 30 (16.8%) were found to be infected with a new species of Cephaline gregarines. Impression smears from the intestine and blood smears of the infected bats showed different shapes of trophozoites and solitary bottle-like gamonts. Semithin sections of the intestinal tract from the infected bats showed different developmental stages of trophozoites and gamonts scattered in the gut lumen and in gut epithelium.

On the other side, also gamonts were detected in the insect vector, *Polyplax brachyrrhyncha*, infesting fruit bats. On one hand, scanning electron microscopy (SEM) revealed that, the fully developed trophozoite was consists of thee merits, on the other hand, transmission electron microscopy (TEM) showed the conical-shaped epimerite was attached to the intestinal epithelium of the host, as well as the gametocyst in the gut lumen and gamonts in caudofrontal syzygy, the associates resembled each other in shape but different in size.

Keywords: Bats; *Rousettus aegyptiacus*; *Polyplax brachrrhyncha*; Gamonts trophozoites; Cephaline; Caudofrontal syzygy

Introduction

Gregarines are known to be extracellular parasites of cavities of invertebrates and lower chordates, though in a minority, the trophozoites remain intracellular. There are types of gregarines, aseptate (acephaline) forms which lack septa, and septate (cephaline) gregarines which have septa dividing the body into segments. These segments are the anteriormost segment, is a modified holdfast organelle or epimerite. The middle segment is the protomerite and the posterior segment is the deutomerite which contains the nucleus. The trophozoites of most trophozoites move in the hemocoel, gut lumen or other body cavities of the host.

Levine in the year of 1988 revised the genus Gregarina and enlisted 13 species [1]. Lipa et al. reported that *Gregarina garnhami*, included in Levine's list, is actually a junior synonym of *G. acridiorum* [2].

Lange and Witenstein described *Gregarina roderosi* n. sp. from the intestine of the grasshopper *Dichrophlus elongatus* as most common in Argentina [3].

The Gregarines and their ultrastructure have been studied by many authors [4-15]. However, in Egypt no published literature about the present species has so far been reported.

Bats are known to be susceptible to many infections for man and animals and they are sheltered close to human dwellings. Their intimate association with man, birds and animals initiates the necessity for the study of their parasites.

During examination of fruit bats in Assiut locality, the present *Gregarina* parasite was encountered so that, the aim of the present study is to reveal the ultrastructure of the new species and to find out its arthropod vectors.

Material and Methods

A total number of 179 fruit bats *Rousettus aegyptiacus* were captured from different localities at Assiut Governorate. The collected bats were examined for the insect ectoparasite, *Polyolax brachyrrhyncha* (Order: Malophaga). The insect was identified according to Galloway and Danks [16] and Palm and Baker [17]. It was dissected and some intestinal smears were prepared and stained with Giemsa stain.

Blood samples from collected bats were examined for protozoan parasites, though thick and thin blood films stained with Giemsa stain. Impression intestinal smears were made also and stained with iron hematoxylin. All measurements were taken by the aid of eye piece micrometer.

Transmission Electron Microscopy (TEM)

Few drops from venous blood and a part of the intestine of the infected bat were immediately fixed in 3 ml of 3% glutaraldehyde solution in phosphate buffer (pH 7.2), for 24 hours and Kept at 4°C in refrigerator. The samples were post fixed in 1% Osmium tetroxide in phosphate buffer (PH 7.2, 300 mom), for 30 minutes. They were washed several times with phosphate buffer solution. The samples were then embedded in Epon which can preserve in structure from distortion during processing then ultra-thin sections were cut by an Ultra microtome and examined by JEOL, 100 CXII operating at 80 KV (TEM).

Scanning Electron Microscopy (SEM)

For scanning electron microscope few drops of blood were fixed in 3% Glutaraldehyde in buffer for 24 hours. Specimens were washed three times in phosphate buffer and post fixed in 1% Osmium tetroxide

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Received September 21, 2015; **Accepted** October 10, 2015; **Published** October 15, 2015

Citation: El Maleck BSA, Abed GH, Maze N, Khalifa R (2015) Morphological and Ultrastructural of a New Species from Cephaline Gregarinidae Infected Fruit Egyptian Bat (*Rousettus aegyptiacus*) and its Vector. J Bacteriol Parasitol 6: 244. doi: 10.4172/2155-9597.1000244

for 2 hours and then washed in the same buffer. They were dehydrated in different grades of ethyl alcohol and then mounted on special holders and coated with gold. Then they were examined in a JSM-T 200 L.V. 5400 scanning electron microscopy (SEM).

Results

The present Gregarina species was encountered in the peripheral blood and intestine of the fruit bat *Rousettus aegyptiacus*.

Out of 179 bats examined, 30 (16.8%) were found to be infected with the new parasite. The earliest uninucleated trophozoites were small in size (≤ 10.5 µm), somewhat ovoid in shape as in Figure 1. Increase in size and gradual development of young trophozoites was accompanied by differentiation of the protomerite-deutomerite septum (Figures 2-4). The epimerite of the unattached young trophozoites appeared relatively large compared to epimerites of older trophozoites. The trophozoites were always solitary not collected (Figure 2), free in the intestinal or gastric lumen of bats or sometimes in the blood. They were showed great variation in length, ranged from 10.5 to 32.625 µm (Table 1).

Gamonts were the most common developmental stages observed (Figure 4). They were small, slender and bottle like shaped and were ranged from 14.25-32.25 µm (Table 2).

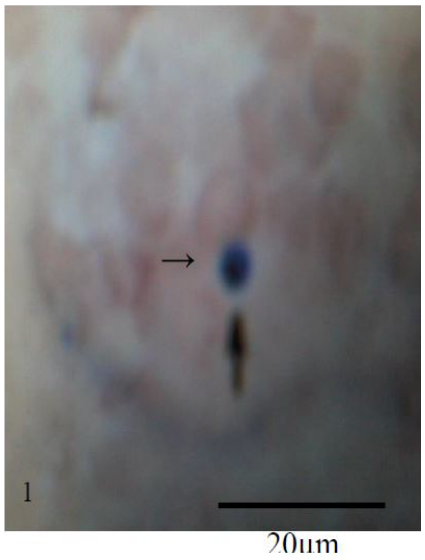

Figure 1: Photomicrograph of blood smear from infected bat showing early ovoid trophozoite stained with Giemsa X=1000.

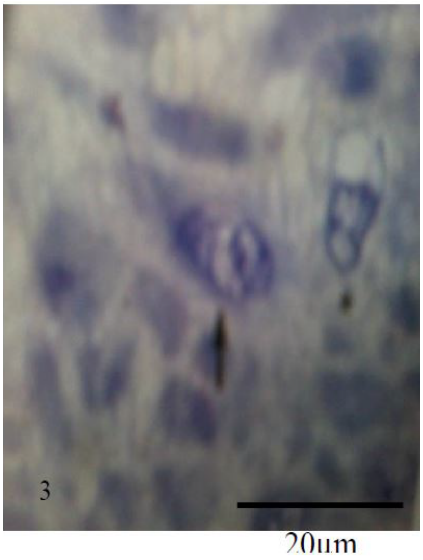

Figure 3: Photomicrograph of semithin section in the intestinal lumen from infected bat showing early stages of trophozoites and fully developed one stained with toluidine blue X=1000.

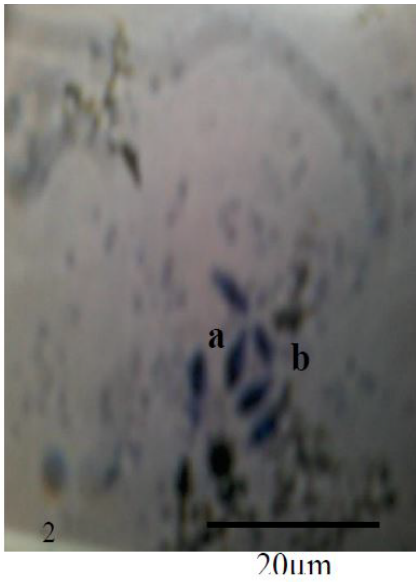

Figure 2: Photomicrograph of intestine smear from infected bat showing a): solitary bottle- like gamont and b) different shapes of trophozotes stained with iron-allum hematoxylin. X=1000.

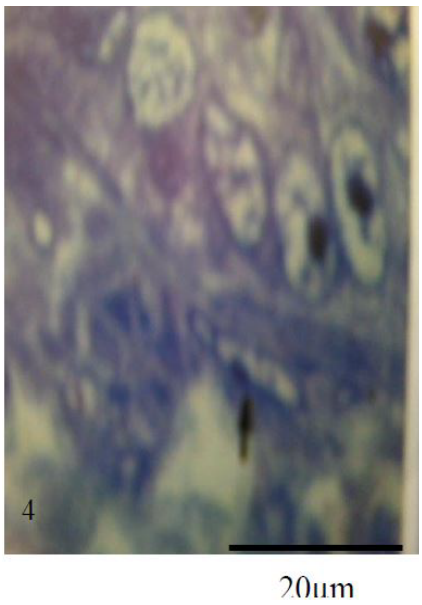

Figure 4: Photomicrograph of semithin section in the intestinal epithelium from infected bat showing slender shaped gamont.

CHARACTER	RANGE	MEAN
Total Length (TL)	10.5-32.625 µm	21.56 µm
Epimerite Length (EL)	1.5-6.75 µm	4.125 µm
Protomerite Length (PL)	1.875-7.5 µm	4.68 µm
Deutomerite Length (DL)	7.5-18.75 µm	13.125 µm
Protomerite Width (PW)	1.5-4.5 µm	3.0 µm
Deutomerite Width (DW)	4.876-8.25 µm	6.56 µm
PL/TL	1 : 5.6-4.3	1 : 4.95
PW/DW	1 : 3.25-1.8	1 : 2.5
PW/PL	1 : 1.25-1.6	1 : 1.4

Table 1: Range and mean measurements of fully differentiated trophozoites (including epimerite, protomerite and deutomerite) of *Gregarina* sp. (n. sp.) infected Egyptian fruit bats (*Rousettus aegyptiacus*).

CHARACTER	RANGE	MEAN
Total Length (TL)	14.25-32.25 µm	23.25 µm
Protomerite Length (PL)	5.625-14.25 µm	9.93 µm
Deutomerite Length (DL)	9.37-18.37 µm	13.87 µm
Protomerite Width (PW)	2.25-6.75 µm	4.5 µm
Deutomerite Width (DW)	3.0-8.25 µm	5.625 µm
PL/TL	1 : 2.5-2.26	1 : 2.38
PW/DW	1 : 1.3-1.2	1 : 1.25
PW/PL	1 : 2.5-2.1	1 : 2.3
TL/DL	1 : 1.5-1.7	1 : 1.6
DL/Dw	1 : 3.1-2.2	1 : 2.65

Table 2: The measurements of gamonts of *Gregarina* sp. (n. Sp.) infected Egyptian fruit bats (*Rousettus aegyptiacus*).

The semithin sections were stained with toluidine blue showed early and fully developed stages of trophozoites scattered in the intestinal contents (Figure 3) and elongated slender gamonts between the intestinal epithelium (Figure 4). At the same time and according to the parasite-vector relationship, gamonts were found in the gut of insect, *Polyplax brachrrhyncha*. They were measured 15 µm in total length, 4.5 µm for the protomerite and 10.5 µm for the deutomerite. It was more slender than those were found in the host (Fruit Egyptian bats) (Figure 5).

Association of gamonts was early, biassociative and caudofrontal syzygy (Figure 6), which was similar to a large extent to conjugation stage. Besides that, fertilization stage represented by two male gametes in their way directed to female one (Figure 7). Scanning electron microscopy revealed obviously that, the three merits of the trophozoites with globular epimerite (Figures 8 and 9).

The deutomerite had granular cytoplasm, nucleus and typical epicytic longitudinal folds (Figure 10). Gamonts were associated in caudofrontal syzygy usually the association was similar in shape but different in size (Figure 11).

Gametocysts in the intestine of the host were spherical or oval in shape and highly variable in size 9- 23.25 µm in diameter, mean 16.125 µm (Figure 12 A). Transmission electron microscopy also revealed that, three merits of trophozoites in the intestinal tract of the infected bats. The epimerits were mostly conical in shape in attached ones (Figure 12B). There was a scar in the protomerite of gamonts where the epimerite was shed (Figure 13).

Discussion

According to the presence of early biassociative gamonts, simple globular or conical epimerite and the other different stages such as, spherical gametocysts, shape of gamont in the intestine of the host and precocious association of gamonts, the present sporozoan cephaline infected *Rousettus aegyptiacus* was affiliated to the genus *Gregarina* (Family: Gregarindae) [1,18,19] .

The present species of *Gregarina* was reported for the first time in fruit bats (*Rousettus aegyptiacus*). Through the natural host for *Gregarina* is known to be grasshoppers or locusts and certain beetles. In the present work the new parasite was supposed to infect the bats through the insect vector (*Polyplax brachrrhyncha*) which live in the skin of the fruit bats.

Detection of *Gregarina* in bats would modify the fact that, *Gregarina* are chiefly parasites of invertebrates and lower vertebrates, supposing that they could be transmitted to vertebrate hosts through the insect vectors.

Lange and Wittenstein reported the ultrastructure of *Gregarina ronderosi* from *Dichroplus elongates* [3]. They found that, the fully developed trophozoites were slender with conical or globular epimerites in attached or unattached forms which measured 10.4-275.1 µm in length but gamonts measured 80-348 µm, while gametocysts 96-376 µm in diameter. However, the three merits of trophozoites with conical or globular epimerite were reported in the present material measured 10.5-32.63 µm and gametocystes 9-23.3 µm in diameter. Accordingly, it was quite evident that, the present species is a different one based on differences of size in some stages. Therefore, the present material is considered a new species to which suggested the name *Gregarina rousetti* n. sp. with the following diagnostic characters:

Type host: *Rousettus aegyptiacus.*

Type locality: El-Badary, Assuit Governorate, Egypt.

Infection site: Intestinal lumen and blood.

Trophozoites: Attached or unattached to gut epithelium, solitary, with great variation in size and epimerite globular in unattached trophozoits and conical in attached one.

Length: 10.5-32.25 µm.

Gamont: Caudofrontal, early association, biassociative, primate and satellite are similar in shape but different in size.

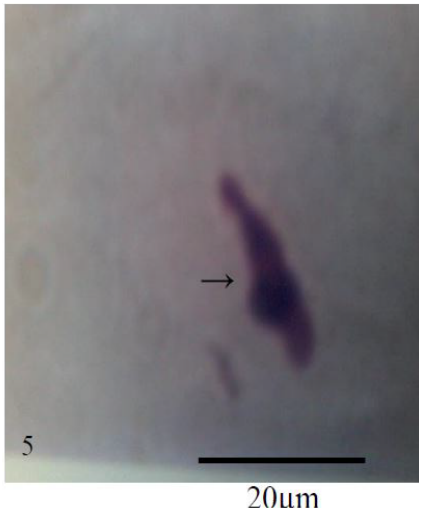

Figure 5: Photomicrograph of impression intestinal smears from insect (*Polyplax brachyrryhncha*) showing gamont stained with Giemsa's stain X=1000.

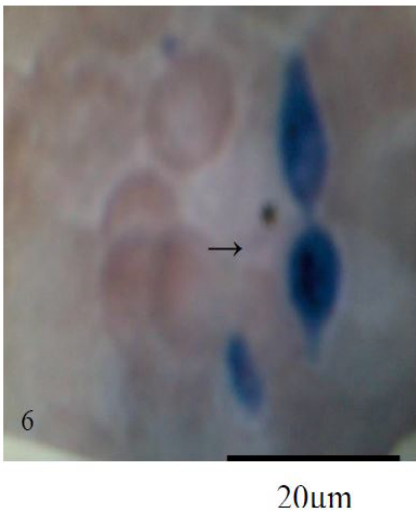

Figure 6: Photomicrograph of blood smear from infected bat showing pairing stage of *Gregarina* sp. (n. sp.) trophozoite stained with Giemsa X=1000.

Size: 9-23.25 μm.

Etymology: The present species was named after the host bat *Rousettus aegyptiacus*.

Deposition of specimen: Zoology department, Faculty of Science, Assiut University.

The present work contributes to the morphology and ultrastructure of these parasites, which may lay a base for further study and related research.

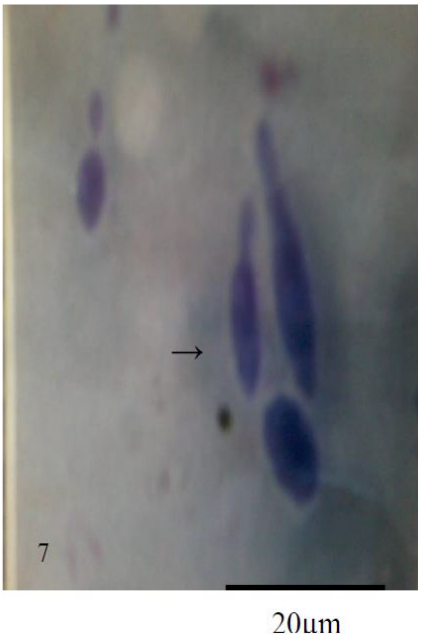

Figure 7: Photomicrograph of intestine smear from infected bat showing fertilization stage of *Gregarina* sp. (two male gametes in their way directed towards one female gamete) stained with Giemsa X=1000.

Figure 8: Scanning electron micrograph of blood smear from infected bat showing the three merits of fully developed trophozote (globular epimerite, protomerite and deutomerite X=5000.

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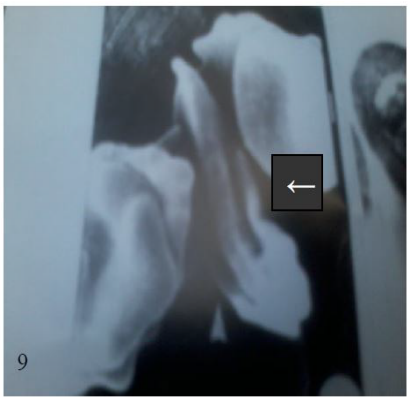

Figure 9: Scanning electron micrograph of blood smear from infected bat showing the three merits of fully developed trophozote X=10000.

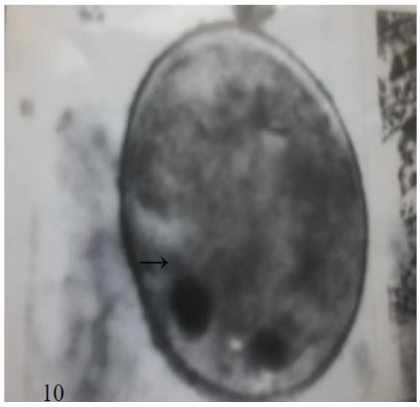

Figure 10: Transmission electron micrograph of blood smear from infected bat showing cross section of gamont of gregarines. X=27000.

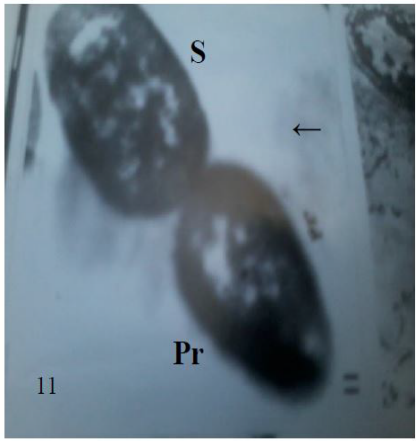

Figure 11: Transmission electron micrograph of blood smear from infected bat showing gamont caudofrontal syzygy (premite and satellite) X=27000.

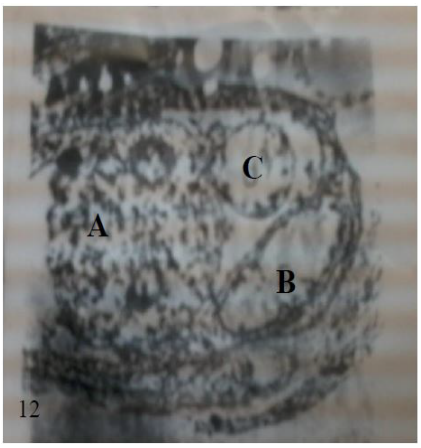

Figure 12: Transmission electron micrograph of intestine infected bat's showing: A-Section of gametocyst in the each intestinal lumen in which associates are still separated and one nucleus X=27000. B-Trophozoite with conical epimerite attached to the intestinal epithelium of the host. C- Cross section of gamont.

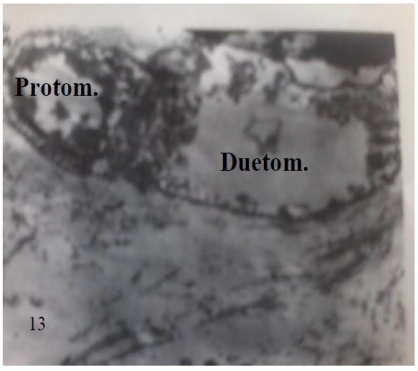

Figure 13: Transmission electron micrograph showing longitudinal section of gamont X=27000.

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