

**Observations on two species of septate gregarine
(Protozoa: Apicomplexa) of the genus Gregarina:
Dufour, from some Useful Insects in
the Local Environment**

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ABSTRACT

The morphology and life history of two species of septate gregarines (Protozoa: Apicomplexa) belonging to the genus Gregarina from insects of the orders Coleoptera and Hymenoptera collected from six cities in the Central Region of Saudi Arabia is given in this communication.

The two species are: 1. G. coccinellae (Lipa) from Coccinella septumpunctata (L.); 2. Gregarina sp. from Apis mellifera (L.) The seasonal intensity of these gregarines and the percentage of infection together with informations about the holotype materials are included.

The holotype materials are at present deposited at the Department of Zoology, Girls College of Education, in Riyadh, Saudi Arabia.

The aim of this work is to study the Protozoa which live in the alimentary canal of useful insects in local environment.

Insects were chosen because of their direct and indirect relationship to man, such as bees and coccinellids. Identification of Protozoa that live in the alimentary canal of insects may be useful in the future for treating such insects and improving their offsprings which can be beneficial to man in agricultural field (Coccinella), and in food industry (like making honey) specially when producing offsprings free from lethal Protozoa.

INTRODUCTION

Dufour (1828) established the genus Gregarina (Protozoa: Apicomplexa) to include a gregarine, Gregarina ovata from the earwig, Forficula auricularia Linn. In their monographs on the cephline gregarines, Watson (1916) and Kamm - Watson (1922) revised the generic characters of the genus Gregarina Dufour as biassociative sporonts, simple globular or cylindrical papilla - like epimerite dehiscence of cysts by sporoducts, and barrel - shaped or dolioform spores. Since the publication of these monographs, workers from different parts of the world have described many species belonging to the genus Gregarina (Hitchcock, 1948; Stejskal, 1955; Oertel, 1965; Lipa, 1967; Kudo, 1971; Amoji and Rodgi, 1976; Halder and Kundu, 1977;

1980, 1981). Levine et al., (1980) made some nomenclature corrections among the septate gregarines and transferred six previously known species belonging to different genera into this genus.

MATERIALS AND METHODS

Two species of insects were collected, these were Coccinella septumpunctata (L.) and Apis mellifera (L.). They were collected during the period between January 1985 to Jan. 1986, from six cities of Saudi Arabia namely Riyadh, Diriyah, Durma, Al-Kharj, Al-Magma'ah and Buraidah. These were examined for their gut parasites on the day of collection. For examination of their gut, the head of the insect was cut and the hind-most segment of the body was pulled out along with the alimentary canal.

Thus separated entire gut was taken onto the glass-slide with a few drops of insects Ringer's solution and smears of the different regions of the gut were prepared. These smears were fixed in Carnoy's fluid, after running through degrading series of ethanol and distilled water, they were stained with ironalum haematoxylin. These stained preparations were mounted in Depex after dehydrated in ascending series of ethanol solution and clearing in butanol and xylene. For the study of intracellular stages, the heavily infected regions of the host intestine were fixed in Carnoy's fluid and paraffin blocks were prepared after dehydration and embedding in benzene paraffin mixtures. Microtome sections were cut at 5-8

um thick on CyroCut microtome maintained at 25C. These sections spread on glass-slides were dewaxed in xylene, stained and mounted as mentioned above. The freshly collected gametocysts were subjected to the moist chamber process for the study of their development and sporulation.

The distribution of carbohydrates was investigated with Best carmine, Alcian blue, PAS and methylene blue-azur II and basic fuchsin.

Nucleic acid was investigated by a Feulgen techniques. Lipid was demonesterated by Oil - Red - O isopropanol method.

RESULTS

The infection of Coccinella septumpunctata (L.) with Gregarina coccinellae (Lipa) is 21% and that of Gregarina sp. is only 8%. The number of gregarines found in each host is also few (5-9). The proventriculus of the host gut is completely devoid of infection where as, the mid gut is the site of infection where all stages of gregarines are found. Occasionally developmental stages of the cysts are found in the hind gut. The cross-sections of the mid gut revealed the intracellular developmental stages of gregarines. The morphology of the two species of gregarines and life history stages of G. coccinellae (Lipa) only are given below.

I. G. coccinellae (Lipa):

Like many other gregarines G. coccinellae (Lipa) (Plate 1) has two major phases in its life cycle, intracellular and extracellular.

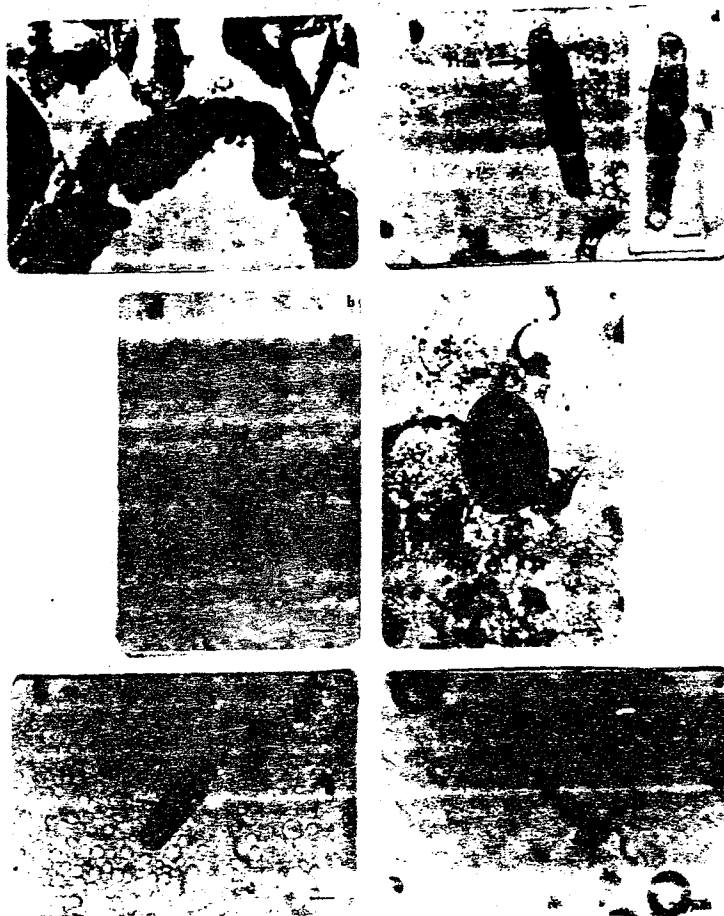


Figure (1): Diagram illustrating the possible developmental cycle of *Gregarina coccinellae* (Lipa) of *Coccinella septempunctata* (L.). Figures have been drawn with the help of Camera Lucida.

- a- Sporozoite.
- b- Earliest intracellular stage in epithelial cell of host mid gut (arrowed).
- c- Advanced intracellular trophozoite.
- d- Adult extracellular trophozoite attached with the epimerite (note the body of the trophozoite is divided by septum (s) into three parts. An epimerite (E) used for attachment; a protomerite midsection (P) and a posterior deutomerite (D) contains a single nucleus (N).
- e- A fully grown trophozoite.
- f- A fully grown sporadin.
- g- Sporadin in syzygy (the interior one, the primitive, the posterior one, the satellite.)
- h- A freshly formed gametocyst with two equal gametocytes.
- i- A fully grown gametocyst.
- j- Asporocyst (oocyst).

The Intracellular Stages:

The young stages of G. coccinellae (Lipa) was first observed within the epithelium of alimentary canal. Each individual is embedded completely in the cytoplasm of an epithelial cell generally rounded at one end tapering gradually at the other end (as shown in plate 1a), its measures 5-12 x 3-9 μm . As it grows further the body divides into two compartments by an ectoplasmic septum. The trophozoite continues to grow within the epithelial cells of the intestine and finally pushes outwards into the lumen to become the extracellular trophozoite but attached to the ciliated epithelial cell with the epimerite.

The Extracellular Stages:

The extracellular stages of G. coccinellae (Lipa) are as follows:

1. The adult trophozoites.
2. Sporadin.
3. Syzygy.
4. The gametocyst.
5. The sporocyst.
6. The sporozoites.

1. The adult trophozoite (Plate 1b):

Detached trophozoites are seen free in the lumen. Epimerite is circular, protomerite is semicircular to flattened wider than long, deutomerite is oval to elongate, it measures 80-134 μm in length.

2. Sporadin (Plate 1c):

The sporadins of G. coccinellae (Lipa) are usually biassociative and measure 80-103 μm in length. Protomerite is semicircular, wider than long. Entocyte granular and opaque. Septum and constriction well seen. Deutomerite is oval to elongate. Nucleus seen as white spot in dark entocyte.

3. Syzygy (Plate 1d):

Generally, two fully-developed sporonts enter into an association to form a syzygium. The general appearance of the associated individuals (primate and satellite) is oval to elongated. The primate is shorter and wider than the satellite. Protomerite in primate is semicircular, wider than long while in the satellite it is flattened few times wider than long. Entocyte granular and darker than that of primate. Septum well seen, while constriction not as clear as in primate. Deutomerite is elongate. Nucleus has three karyosomes and is seen as white spot in dark entocyte.

4. The Gametocyst (Plate 1e):

It is oval up to 115 μm in length. The cyst is covered externally by an enveloping wall which is uniform around. Further stages of development of gametes and zygotes within the gametocyst were not observed.

5. The Sporocyst (Plate 1f):

A Mature gametocyst contains a large number of sporocysts were observed. Spores are spindle-shaped 7 μm wide and 12 μm long and occurring solitary.

6. The Sporozoite:

Sporocysts contain unknown number of sporozoites. The possible developmental stages of life cycle of G. coccinellae (Lipa) is shown in Fig. (1).

II. Gregarina sp:

Because of rarity of infection, I could not follow up the life history or recognise the species of this gregarine. Only two stages were seen:

1. The Intracellular stage.
2. Sporadin.

1. The Intracellular stage (Plate 2a & b):

The young stages of Gregarina sp. was first observed within the epithelium of the host mid gut as revealed in sections. Each individual is embeded completely in the cytoplasm of an epithelial cell generally rounded at both ends. Its measures 5-10 x 3-8 um.

2. Sporadin:

The sporadins of Gregarina sp. are characteristically solitary and measure 20-29 um in length (Plate 2c). Protomerite is wide oval. Entocyte homogenous and translucent. Septum is well seen. Deutomerite is cylindrical and rounded at its posterior end. Nucleus is circular with one karyosome. Gametocysts and sporocysts are unknown.

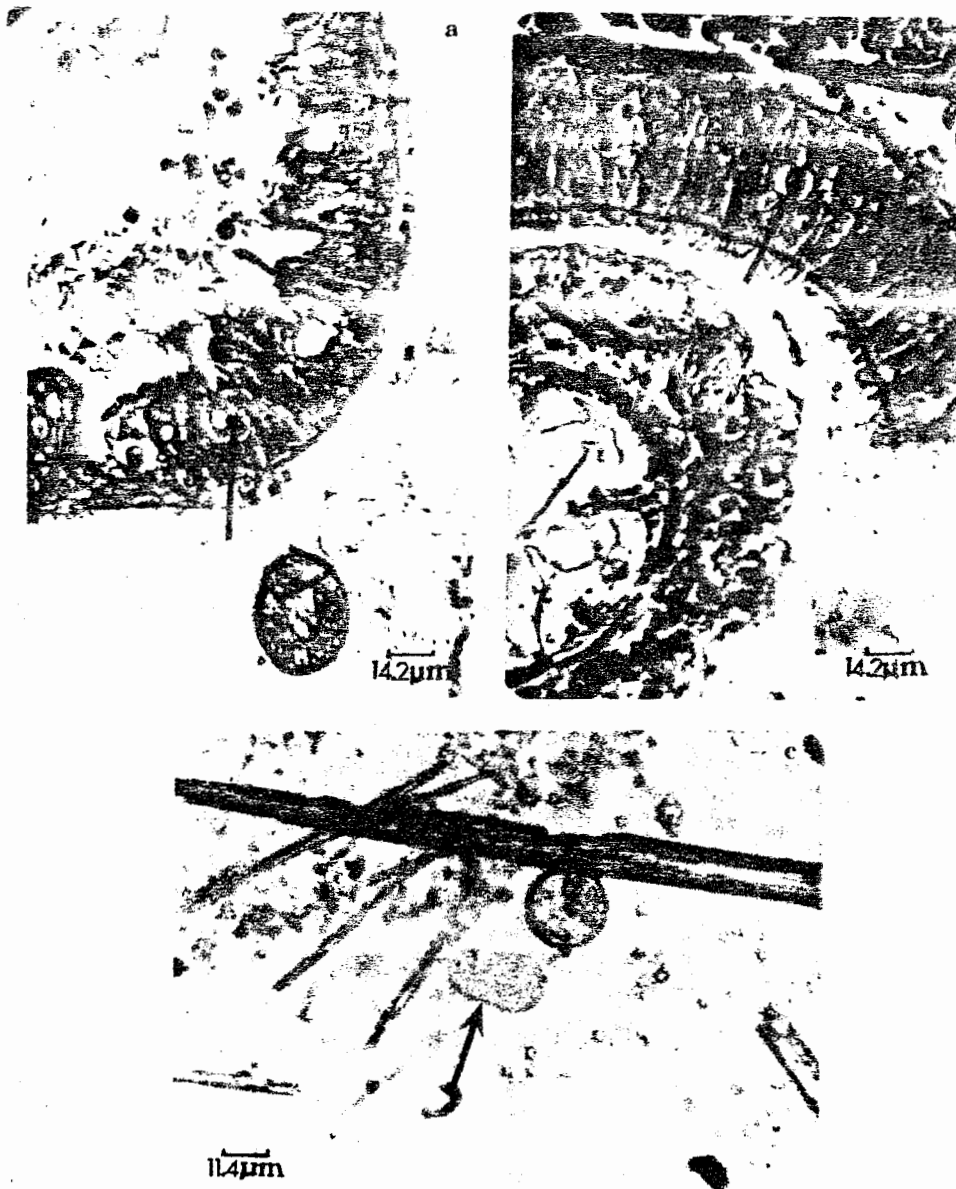


Plate 2:

a&b: Transverse sections of the gut of *Apis mellifera* (L) showing intracellular stages (arrowed) of *Gregarina* sp. (Haidenhain's iron haematoxylin).

Light micrographs.

c: Photomicrograph of *Gregarina* sp. (arrowed) from the gut of *Apis mellifera* (L) (Haidenhain's iron haematoxylin).

DISCUSSION

In Saudi Arabia there is no work that has been done on Protozoa parasites of insects, except the discovery of a new species of gregarine Stylocephalus arabica from Adesmia cancellata Klug (Koura and Al Jubair, 1989).

The present study includes findings resulting from the examination of two species of insects belonging to two different orders Coleoptera and Hymenoptera, the two species of insects are: Coccinella septumpunctata (L.) and Apis mellifera (L.).

Lipa (1967) recorded G. coccinellae in Coccinella Septumpunctata but did not follow its life cycle. The maximum length of G. coccinellae described in the present study is 134µm while 179 µm by Lipa, the percentage and intensity of infection are much less than that described by Lipa. Sporocysts are similar to that found by Lipa but not in chain.

Gregarina sp. that found in Apis mellifera is different in shape and size than that described by Hitchcock (1948), Stejeskal (1955), and Oertel (1965).

Because of rarity of infection it was difficult to classify the species or follow its life cycle.

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