

A New Septate Gregarine (Apicomplexa: Sporozoa) from an Agricultural Pest of Imphal Valley, Manipur, India

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Abstract: Descriptions of a new species of septate gregarine i.e. *Didymophyestriangulogametus* sp. nov. from the midgut of *Chondracis rosea* (Insecta, Orthoptera, Acrididae), is provided. Trophozoites attain a length of 39.9µm -167.7µm and their protomerite is more or less subconical, measures 49.8µm -68.8µm at an average and deutomerite is elongated or rectangular in shape and measures 33.6µm-120.4µm. Gamonts are solitary and spherical, Gametocysts are 140µm-292µm. Three gametocysts are joined by thin ectocyst forming a triangle like structure. The morphological details supported with photomicrographs are provided in the manuscript.

Keywords: Apicomplexa, *Didymophyes*, new species, Manipur, India

1. Introduction

During the course of an investigation on the protozoans of agriculturally important insects of Manipur, species of Septate gregarines under the genus *Didymophyes* [1] are encountered. The genus *Didymophyes* is characterized by the presence of a small pointed epimerite, spherical cyst that dehiscence by simple rupture and ellipsoidal spores. *Didymophyes gigantean* is the type species of the genus. Léger [2] after studying the parasites described earlier [1] notes that the satellite of the biassociative forms are without protomerite. He, therefore, creates a new family Didymophyidae for these gregarines that have two to three sporadins in association and the satellite without septum. The present communication records a new species belonging to *Didymophyes* [1] under the family Didymophyidae [2] from Manipur, India.

2. Material and Method

2.1 The Research Time and Place

The work is carried out in Imphalwest district of Manipur, India, between June 2013 to December 2014.

2.2 Research Material

Insects were collected from different localities with the help of nets. The collected insects are brought alive to the laboratory. They are dissected under a dissecting microscope in 0.9% saline solution and examined for their protozoan parasites. A thin film of mid gut fluid is drawn out on a slide, covered with a cover slip for examination of living protozoans under a phase contrast microscope. After the initial study of the living protozoans, the content of the midgut is semi-dried and fixed in Schaudin's fluid. The smears are stored in 70% ethyl alcohol for removal of mercuric chloride. The slides are then passed through a descending series of alcohol and lastly in distilled water. Then, they are transferred to 3% iron alum solution and stained with Heidenhain's haematoxylin solution, then by dehydration in an ascending series of alcohol, cleared in xylene and mounted in Canada balsam. Cysts are collected

from the hind-gut of infected hosts and cultures in moist chamber for sporulation according to Sprague [3]. Photomicrographs are taken under an Olympus phase contrast microscope with an Olympus camera (Model GE-52TRH). All the measurements are taken with the aid of calibrated ocular micrometers (µm) according to Clopton [4].

2.3 Measurements

Measurements of the specimen are made according to Clopton [4].

3. Result

3.1 Observation

The morphometric and photomicrographs of *Didymophyestriangulogametus* sp. nov. are presented in Fig. 1 and Table 1.

- **Development:** No intracellular development stages formed.
- **Trophozoite:** The trophozoite has an elongated or rectangular body shape measuring 39.9-167.7µm (65.53±29.29) in length. Epimerite not observed. The protomerite is more or less subconical, always broader and with a slight concavity in the middle of its anterior side. It measures 22.3-47.2µm (40.91±6.0) × 49.8-68.8µm (59.2±5.84) in average. The deutomerite is elongated or rectangular in shape and measures 33.6-120.4µm (69.45±31.5) × 52.1-81.7µm (52.2±5.93) in average. Its posterior extremity is curved inwards. The cytoplasm of the deutomerite is more granulated than the other portions of the body. The nucleus is elliptical in shape, may be situated anywhere in the deutomerite and it measures 16.9-21.5µm (18.9±1.91) × 1-4.3µm (2.9±0.97) in average. It possesses distinct nuclear membrane and large endosome. The pellicle is very delicate and the epicyteal striations are not clearly discernable. The septa separating the protomerite from the deutomerite are clearly observed.
- **Sporadin:** The sporadin is oblong or rectangular in shape. It measures 73.5-133.3µm (105.7±26.2) in length. The protomerite of the sporadin is somewhat bent at the

anterior portion and it measures $24.5-34.4\mu\text{m}$ (26.7 ± 5.3) \times $41.8-60.2\mu\text{m}$ (48.2 ± 5.89) in average. The deutomerite is rectangular or cylindrical in shape. The septum separating the protomerite from the deutomerite of the sporadin is very thick. The deutomerite measures $80.7-98.9\mu\text{m}$ (89.7 ± 5.96) \times $101.2-120.4\mu\text{m}$ (105.83 ± 6.7) in average.

- **Association:** Solitary as well as biassociative. The association is always caudofrontal in nature. During the beginning of association, the primate in most of the case cannot bear an epimerite in it. The deutomerite of the primate has a long rounded posterior extremity. The satellite, as a rule present a protomerite and its deutomerite is cylindrical in shape. A thick band of cytoplasm connects the primate with the satellite.
- **Gametocyst:** Freshly collected gametocysts from the hind gut of the host are dark brown in colour, and spherical bodies enclosing three gametocysts are joined by thin thread and form a triangle like structure. The three gametocysts are more or less equal in size. It measures $140.9-292.4\mu\text{m}$ (204.4 ± 44.18) in diameter at an average. Inside the moist chamber, the joining between the gametocyst disappears after 32 hour of development and at 69 hour it dehisces by normal rupture liberating the spores.
- **Spore:** The spore are smooth, ovoidal in shape with remarkably double spore wall. The spores measure $8.6\mu\text{m}$ \times $12.9\mu\text{m}$ in average. When stained with Lugol's iodine solution, eight globular sporozoites are closely seen in regular fashion inside each spore.
- **Prevalence:** 35 out of 75 (46.6%).

4. Discussion

In the presence of Sporadins in association and Satellite without septum, the present gregarine is placed under the genus *Didymophyes* [1] under the family Didymophyidae [2]. It comes close to *Didymophyes ridigus* [5] in having a concavity in the proximal end of the protomerite of the trophozoite. In the ratios of the LP and TL, it also shows affinity with *Didymophyes tridactylae* [6]. But the present species differs from these species in all other aspects like shape of protomerite, size of gametocyst and arrangement of sporozoites in spores. *Didymophyes ridigus* has characteristic disc-like epimerite with prominent ridges radiating, posterior end of the primate firmly fits in the cup-like depression at the anterior end of the satellite, rectangular protomerite, gametocyst $49\mu\text{m}$ \times $46.6\mu\text{m}$. And in *Didymophyes tridactylae*, the trophozoite is vase-shaped, its epimerite hyaline papilla like structure, a thick band of cytoplasm connects the primate with the satellite. Deutomerite spherical, Gametocyst $116-233.2\mu\text{m}$ \times $133.3-283.2\mu\text{m}$, with an oval ectocyst measuring $33.3\mu\text{m}$ in diameter, ovoid spore surround by a hyaline rectangular coat. The present species is most peculiar in having ectocysts and shows attachment of three gametocytes in the form of a triangle like structure and in the presence of ellipsoidal spore. It is therefore,

considered as new to science for which the name *Didymophyes triangulogametussp.nov* is proposed.

5. Conclusion

The systematics of the present new species of septate gregarines have been determined by certain parameters like measurements of trophozoites or gamonts, their ratios as well as the peculiar gametocyst having attachment forming triangle like structure and the type of hosts. The infection are observed from *Chondracis rosea*. However, it is not known whether the infected hosts recover the damages after the release of the intracellular parasites into the host gut lumen. There is need to study the host parasite interaction to have clear knowledge about the damage of the insect hosts due to the gregarine infection which may throw some light towards the possible role of gregarines on the biological control of the insect pests.

6. Acknowledgement

The specific name has been given to emphasize the shape of the gametocyst of the gregarine.

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Figure-1

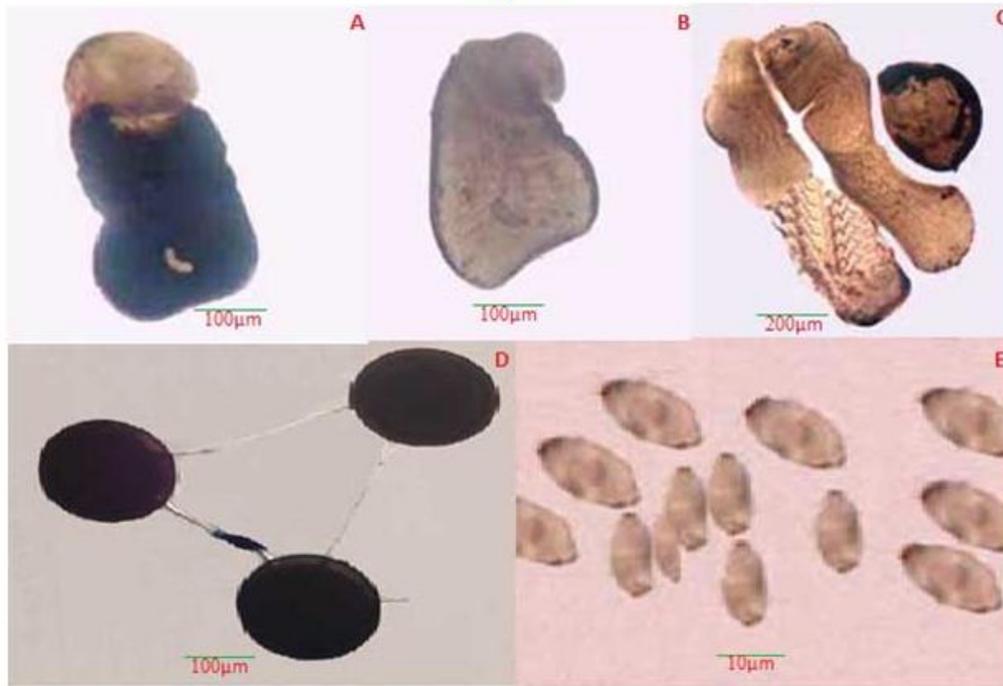


Fig1: Photomicrographs of the *Didymophyes triangulagametus* sp. nov. A,B-Trophozoite, C- A group of Syzygy D- Gametocysts, E- Spore.

Table 1: Showing the comparative characters of *D. rigidus*, *D. tridactylae* and *Didymophyes triangulagametus*

| Characters | <i>D. rigidus</i> , Ghose <i>et al</i> , 1987 | <i>D. tridactylae</i> , Kundu <i>et al</i> , 1987 | Present Study |
|--------------|---|---|---|
| Total length | 94.5µminlength | 34.0-157.0 µm | 39.9-167.7 µm in length |
| Epimerite | Papilla-like, it becomes disc-like with prominent ridges radiating from central area when viewed from top | Hyaline papilla-like 4.3-12.8µm in length | - |
| Protomerite | Rectangular or hemispherical and is broader than long | Rectangular in trophozoite hemispherical in sporadins | Protomerite is more or less subconical, always broader and with a slight concavity in the middle of its anterior side |
| Deutomerite | Posterior end of the primate firmly fits in the cup like depression at the anterior end of the satellite. Deutomerite of the primate is rounded while that of the satellite is gradually narrower | Deutomerite is more or less spherical in shape | Deutomerite is rectangular in shape |
| Nucleus | Ovoidal to elliptical | Spherical or ovoidal | Elliptical |
| Sporadin | Solitary, biassociative ovoidal to cylindrical | Bi-associative, unusual sporadins with broad flat protomerite | Bi-associative; rectangular in shape |
| Gametocyst | Spherical | Ovoidal, with ectocyst | Spherical, enclosing three gametocysts are joined by thin ectocyst and form a triangle like structure |
| Spore | Ellipsoidal | Ovoidal, double walled with rectangular hyaline coat | Ovoidal, double wall |
| LP:TL | 1:4.0-14.5(8.02) | 1:3.5 | 1:3.5 |
| WP:WD | 1:1.0-30(1.96) | 1:1.3 | 1:1.2 |
| Host | <i>Oryzaephilummercator</i> | <i>Tridactylus</i> sp | <i>Chondracis rosea</i> |
| Locality | Kalyani, West-Bengal, India | Kalyani, West-Bengal, India | Manipur, India |