



## Protozoan Parasites of Some Grasshoppers of Manipur, India.

### KEYWORDS

Manipur, Grasshopper, Gregarine, Species.

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### ABSTRACT

Protozoan parasites infest a wide range of Arthropods including Insects like Grasshoppers. During the course of a survey in Manipur, Grasshopper were collected and examined for protozoan parasites. Gregarines were found from the mid-gut region. The taxonomy of Gregarine Species are based on the trophozoite morphology, epimerite morphology, timing of gamont association, gametocyst morphology and the method of gametocyst dehiscence (Clopton 2009; Léger 1892; Smith and Cook, 2008). In the present Communication five species of Septate gregarine trophozoites belonging to *Brustiospora*, *Lepismatophila*, *Retractocephalus*, *Nematopsis* and *Amphiplatyspora* genera are reported from three species of grasshoppers found in Manipur. The morphological details supported with photomicrographs are provided in the manuscript.

### Introduction

Protozoa are a diverse and poorly understood group of unicellular organisms. Among them apicomplexans parasites inhabit the intestines, coeloms and reproductive vesicles of marine, freshwater and terrestrial invertebrates. Of 6,000 described apicomplexan species, 1,600 are gregarines and many more are estimated to exist (Adl et al. 2007; Hausmann, Hülsmann, Levine 1988; Morrison, 2009; Perkins et al. 2000). The description of gregarine species has historically been based on the morphological species concept that emphasized features like trophozoite morphology, epimerite morphology (mainly septate eugregarines), timing of gamont association, gametocyst morphology, and the method of gametocyst dehiscence (Clopton, 2009; Léger, 1892; Smith and Cook, 2008). Host association has also been used as a taxonomic criterion because many gregarines are inferred to be stenoxenous, being able to mature in only a single host genus or species (Levine, 1979; Perkins et al 2000). Nonetheless, some gregarine species can infect different host species, and one host species can be infected with several different gregarine species (A'bro, 1974; Rueckert and Leander, 2009). Moreover, the shape, size, and ultrastructure of trophozoites are often highly variable depending on different developmental stages of the parasite and host environmental conditions. In the present Communication five species of Septate gregarine are reported.

### Material and Methods

The Adults of *Oxya hyla hyla*, *Chondracis rosea* and *Choreodocus robusta* were collected from various grass fields of Manipur (24° 44' N, 93° 58' E) in the morning between 6 to 8 a.m. with the help of net, were kept in glass tubes and brought alive to the laboratory for investigation from April, 2011 to December, 2014. These were decapitated, their guts carefully dissected out under a dissecting microscope and gently pressed to expel the parasites from the gut lumen. Thin smear preparations were fixed in Schaudinn's fixative and subsequently stained with Heidenhain's haematoxylin (Kudo, 1966). Gametocysts were recovered from the hind gut and placed in moist chambers (> 80% relative humidity) for sporulation (Sprague, 1941). The structure of the oocysts were studied by using Lugol's iodine solution. Figures of stained specimens were drawn with the aid of a camera lucida. Measurements of fresh

materials were taken using an ocular micrometer calibrated with a stage micrometer. All measurements, unless otherwise mentioned, are in micrometers. Forty specimens each of mature gamonts and associations were randomly measured from the infected hosts. Similarly, thirty gametocysts and fifty individual oocysts were measured. Measurements were taken from widest part of protomerite, deutomerite, nucleus, gametocyst and oocyst and are presented in this paper as range values, followed by means, standard errors and sample sizes in parentheses. Blue filters were used for measurements and daylight filters were used for observation of colour in living specimens. Nomenclature for shapes used in this manuscript conforms to (Clopton, 2004).

### Results and discussion

Several motile trophozoites were observed in the midgut of infected *Oxya hyla hyla*, *Chondracis rosea* and *Choreodocus robusta*. The specimens show five forms of trophozoites i.e. Trophozoites of *Brustiosporaindicola*, *Lepismatophila cruzi*, *Retractocephalus aulacophorae*, *Nematopsis annulipes* and *Amphiplatyspora striata* as shown in Figure 1, Plate 1.

#### ***Brustiosporaindicola* Kundu and Haldar, 1981**

Measurement: Table.1.

Description: The trophozoite were encountered from within the lumen of the host's midgut. The body is elongated and measures 68.2µm-146.7µm (121.6). The protomerite is more or less cylindrical and it measures 6.52µm-27.9µm × 9.0µm-29.7µm (20.7). The deutomerite is elongated and measures 36.7µm-118.8µm (98.4) × 11.7µm-42.5µm (20.4), gradually narrows to a rounded posterior extremity. The pellicle is very thin and the cytoplasm is uniformly granulated. The nucleus is almost spherical, situated just behind the protomerite - deutomerite septum and measures 6.2µm in diameter.

Host: *Oxya hyla hyla*

Habitat: Mid gut and hepatic caeca

Incidence: 3 out of 7 are infected

***Lepismatophila cruzi* Kundu and Haldar, 1984**

Measurement: Table.1.

Description: The detailed structures of a trophozoite measures 35.9µm-149.0µm (82.8). The body as usual bears epimerite, protomerite and deutomerite. The epimerite is petalloid or has two horned structure that pushes through the host epithelium. The protomerite is sub-conical or hemispherical in shape, measures 6.4µm-24.0µm (15.1) × 49.9µm-11.7µm (28.1). It is followed by the deutomerite and measures 125µm-45µm (69.5) × 15.0µm-54µm (30.2). It is highly granulated and is separated from the protomerite by a clear septa. The nucleus is circular and measures 12.7µm in diameter, lodged immediately behind the septum, possesses a distinct nuclear membrane and encloses a big endosome and few chromatin granules in it.

Host: *Chondracis rosea*

Habitat: Hepatic caeca

Incidence: 4 out of 8 are infected

***Retractocephalus aulacophorae* Haldar, Chakraborty and Kundu, 1982**

Measurement: Table.1.

Description: The intracellular form of the parasite is somewhat oval in outline. Its cytoplasm appears opaque white when observed in fresh smear preparation. It may reach upto a length 139µm -309µm (154.1). The protomerite is somewhat conical in shape and is pointed towards the apex and measures 10.9µm-65.0µm × 26.0µm-97µm (39.9). The deutomerite is the largest segment of the body and is separated from the protomerite by a deep constriction. The deutomerite is obese in shape, and is broadest slightly behind the septum, measure 123µm-244µm (150.7) × 18.7µm-139µm (43.6). It tapers posteriorly and ends in a rounded proximity. The nucleus is spherical, situated almost at the center of the deutomerite and measures 7.5 µm in diameter.

Host: *Oxya hyla hyla*

Habitat: Mid gut and hepatic caeca

Incidence: 5 out of 13 are infected

***Nematopsis annulipes* Prasad and Janardanan, 2001**

Measurement: Table.1.

Description: Development extracellular, smallest observed trophozoite, narrow, elongate and ovoid. It may reach up to 198.7µm-315µm (272.7). Protomerite shape subspherical to ovoid, measures 29.7µm-64µm (38.4) × 11.7µm-48µm (29.7). Epicyte uniformly thick and striated. Endocyte granulated. Septa circular, convex towards deutomerite. Deutomerite narrow behind septum, gradually dilates, posteriorly end broadly round or flat and measures 11.9µm-252µm (127.7) × 33.2µm-72µm (49.4); nucleus spherical, variable in position and measures 7.5µm in diameter.

Host: *Choreodocus robusta*

Habitat: Mid gut

Incidence: 6 out of 21 are infected.

***Amphiplatyspora striata* Kundu and Haldar, 1984**

Measurement: Table.1.

Description: The trophozoite encountered in the smear preparation is cylindrical in shape, with a large ovoidal protomerite and elongate deutomerite. The trophozoite varies markedly in shape and size in both protomerite and

deutomerite. It measures 110µm-411.9µm (255.5). The trophozoite has a globular, fusiform, rectangular, semi-lunar or sickle-shaped, hemispherical or a hat shaped, Protomerite measuring 22.9µm-79.7µm (41.9) × 29.7µm-158.9µm (79.1). The deutomerite is cylindrical flask shaped, vermiform, cylindrical-conical with broadly rounded anterior and gradually tapering posterior extremity and pitcher shaped body measuring 79.1µm-332.2µm (220.7) × 29.8µm-169µm (89.7). The nucleus is situated at the deutomerite portion and measure 17.5µm in diameter. The longitudinal striation converge at a point near the posterior tip of the body. The various shape of the protomerite and deutomerite are probably due to the presence of such strong striations in the body.

Host: *Chondracis rosea*

Habitat: Mid gut and hepatic caeca

Incidence: 4 out of 18 are infected

In the course of the present investigation 20 *Oxya hyla hyla*, 26 *Chondracis rosea* and 21 *Choreodocus robusta* were examined of which 8, 8 and 6 respectively were infected by eugregarines. The present findings showing a number of septate gregarines from the Grasshoppers of Manipur, is an added knowledge to the study of the biodiversity of protozoan groups.

The present specimens of *Brustiospora indicola* shows similarity in the size and shape of Protomerite, Deutomerite, Gametocyst, Spore, with the specimens of (Kundu and Haldar, 1981). But there is slight variation in the total length of the Trophozoite. The present specimen is also reported from a new host i.e. *Oxya hyla hyla*. The host are obtained specially in the months of September to November and the infection is carried mostly during these months. An average 42% are infected by the parasite.

On the other hand *Lepismatophila Cruzi* was originally described by Kundu and Haldar in 1984 from Silver fish of West Bengal. In the present work it has been described from *Chondracis rosea*. The morphological details and measurements are within the range described in the original work. 50% of the host examined were infected with the parasite. *Retractocephalus aulacophorae* is another gregarine that is prevalent in grasshoppers. In the original description of Haldar, (Chakraborty and Kundu, 1982) it has been described from the midgut of the Beetle, *Lema* sp. The present specimen is described from *Oxya hyla hyla*. The Trophozoite is a little smaller in the present specimen. The ratio of LP:TL and WP:WD show much similarity. In the presence of intracellular developmental phase, Simple globular epimerite and barrel shaped spore they are much similar. The parasite availability coincides with the abundance of the host i.e. during the months of June to September, 39 % of the hosts are infected with the parasite during this period. Another gregarine *Nematopsis annulipes* has been recovered from *Choreodocus robusta* while the original specimens of (Prasad and Janardanan, 2001) were from the Crustacean *Uca annulipes*. The prevalence rate in the present case is much higher than the original. In addition the size range of the present specimen are much higher. In other morphological details like those of Sporadins, Epimerite, Protomerites, presence of satellites, structure of Gametocysts there are close similarities. From *Chondracis rosea* another gregarine *Amphiplatyspora striata* had been recovered. (Kundu and Haldar, 1984) described the species from an Orthopteran Insect, *Pteronemobius concolor*. Comparison of the morphological characteristics and measurements show close resemblance

with the present specimens. The intensity of infection is low ranging around 27% which is close to 22% in case of the original specimen.

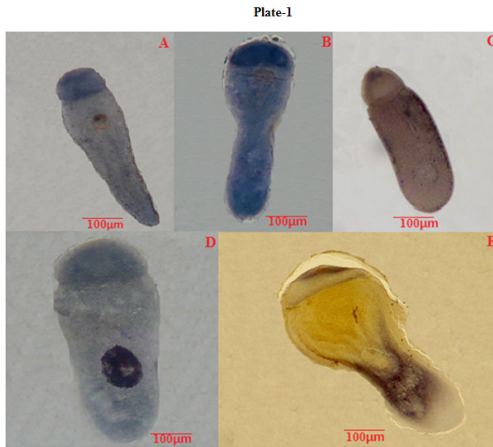


Figure 1. Photomicrographs of the different septate gregarines A-Trophozoite of *Brustiospora indicola*, B-Trophozoite of *Lepismatophila cruzi*, C-Trophozoite of *Retractocephalus aulacophorae*, D-Trophozoite of *Nematopsis annulipes*, E-Trophozoite of *Amphiplatyspora striata*

Sporadin	Typically Solitary	Solitary	Biassocia-tive	Association of three Sporadin	Solitary
Gametocyst	Elliptical or egg shaped	Bean-Shaped with one side convex	Spherical	Spherical to Ovoid	Ovoidal with prominent ectocyst: dehisces by simple rupture
Oocyst	Spherical	Boat-Shaped	Barrel-Shaped	Spherical uninucleated	Spore cylindrical with polar thickenings: extruded in chains
LP:TL	1:5.0	1:6.2	1:4.7	1:4.9	1:5.2
WP:WD	1:1.0	1:1.7	1:1.3	1:1.5	1:0.9
Host	Oxya hyla hyla	Chondracis rosea	Oxya hyla hyla	Choreo-docus robusta	Chondracis rosea
Locality	Manipur, India	Manipur, India	Manipur, India	Manipur, India	Manipur, India

Table: 1. Measurements of Five Species of Septate gregarines

Characters	Brustio-sporaindi-cola, Kundu and Haldar, 1981	Lepis-matophi-la cruzi, Kundu and Haldar, 1984	Retracto-cephalus aulacopho-rae, Haldar et al. 1982	Nematop-sis annulipes, Parasadan and Janardan, 2001	Amphi-platys-pora striata, Kundu and Haldar, 1984
Total length	68.2µm-146.7µm	35.9µm-149.0µm	139.0µm-309µm	198.7µm-315µm	110µm-411.9µm
Protomerite	More or less Cylindrical	Sub-Conical or hemispherical	Somewhat Conical	Subspherical to Ovoidal	Sickle shaped, hemispherical or hat shaped
Deuteromerite	Elongated and gradually narrows to posterior extremity	-	Obese, distinct epicyteal striations	Rounded to flat	Cylindrical flask Shaped
Nucleus	Spherical	Circular	Spherical, one or two Karyosomes	Spherical	Spherical to semi lunar

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