

Didymophyes Bayaramae sp.nov., A New Septate Gregarine (Protozoa: Apicomplexa) from *Aphis fabae* of Manipur

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Abstract

The protozoa septate gregarines infect the midgut of *Aphis fabae* and cause severe infection to the intestinal tissues of the host. During a biodiversity survey on the septate gregarines parasite by the Aphid groups, we collected specimens of *Aphis fabae* from a bean farm in Imphal, Manipur, India. We dissected the host and isolated some protozoan parasites from the host's midgut. We encountered a new species of septate gregarine, *Didymophyes jayaraman* sp.nov. Its growth in pairs characterizes the protozoa. Its body parts have epimerites in the anterior body, protomerite in the middle body, and a large deutomerite in the posterior body. The protozoa have a total length of 142.8µm in length. Its trophozoite stage is vase-like; epimerite is papilla-like and becomes disc-like, with prominent ridges radiating from the whole mouth to the top of the protomerite. The protomerite is conical, and the deutomerite is more or less spherical, with a nucleus at the posterior portion. The shape of the sporadin is straight with hemispherical or egg-shaped protomerite, broader than long and ovoidal in shape. Protomerite is ellipsoidal to rhomboidal in shape. Gametocytes are round, and spores are spherical. The species differs from closely related species *Didymophyes rigidus* in the shapes of protomerites, deutomerites, gametocytes, and spores. It also differs from *Didymophyes berhamporensis* in the shapes of trophozoites, epimerites, protomerites, deutomerites, sporadins, gametocytes, and spores. The prevalence of the infection was 25 out of 60 (33.3%). In the present article, we describe the distinctive characteristics of *Didymophyes bayaramae* sp.nov and its taxonomic position. We are providing photomicrographs of the parasite at different stages.

Keywords: Septate gregarine, *Aphis fabae*, *Didymophyes bayaramae*.sp.nov. and Manipur.

INTRODUCTION

The Hemipterans insects like aphids, in association with other arthropods, cause immense damage to the field of agriculture. Frequently, gregarines parasitized these Hemipterans in their hepatic cancer and midgut. Occasionally, protozoans cause significant damage to the gut epithelium of their hosts. As a result, these parasites are closely studied as potential agents for the biological control of harmful insects. [1–3]. Among the Apicomplexa, the diversity of the gregarines is surpassed only by the coccidia. Eugregarinida are all parasitic, but they are absent in invertebrates. Few workers reported a few species from tunicates. Eugregarinorida contains 1656 species divided among 244 genera; however, these figures are, at best, a cursory survey of the group's total biodiversity. Scientists who worked on Gregarines reported from only about 3,124 invertebrate species, less than one-third of one percent of the named invertebrate fauna [4]. They reported

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gregarine species from insects, the most deceptive invertebrate group. The septate gregarines, also called the cephaeline gregarines, belong to the phylum Apicomplexa and the class Conoidasida under the sub-kingdom Protozoa. *Aphis fabae* (Insecta: Hemiptera) is a common pest occurring in bean farm plants in Manipur. Occasionally, the damage caused by these insects is alarmingly high. Therefore, we conducted a study to determine if these organisms had any internal parasites in their guts and, if so, whether these parasites aided the hosts in digesting the food consumed by these pests.

Interestingly, we know that septate gregarines parasitize the hosts collected from all the sources (Apicomplexa: Sporozoa), with infection reaching about (33.3%) at times. Upon preliminary examination, we identified the gregarines of the genus *Didymophyes* [5]. However, a detailed study of their morphology and life history has shown that these species differ depending on the bean farm item infected by their hosts. The present communication deals with the structure and life history of the species of septate gregarines infecting hemipterans insects. The information might help future investigations using these protozoans for the biological control of insect pests.

The ratios used in this paper are those of the length of protomerite to the total length (L.P.: T.L.) and the width of the protomerite to the width of deutomerite (W.P.: W.D.). We used the following abbreviations in this paper: L.E. - Length of epimerite; L.P. - Length of protomerite; L.D. - Length of deutomerite; L.N. - Length of the nucleus; T.L. - Total length; WE - Width of epimerite; W.P. - Width of protomerite; W.D. - Width of the deutomerite; W.N. - Width of the nucleus.

MATERIALS AND METHODS

We collected the insect hosts from the Imphal-West district and brought them alive in the laboratory. We dissected the host under a dissecting binocular microscope in 0.9% saline solution and examined for the presence of protozoan parasites. We extracted a thin fluid film from the mid-gut and placed it on a slide with a cover slip to examine living protozoans using a Phase Contrast Microscope (Olympus CX41). After initially studying the living protozoans, we semi-dried the midgut contents and fixed them in Schaudinn's fluid for 20 minutes. We then stored the fixed smears in 70% ethyl alcohol to remove mercuric chloride. The slides were passed through a descending series of alcohols for 5 minutes each, followed by placement in distilled water. Next, the slides were transferred to a 3% iron alum solution overnight and stained with Heidenhain's hematoxylin solution for 20 minutes. The preparations were thoroughly washed, dehydrated in an ascending series of alcohols, cleared in xylene, and finally mounted in Canada balsam. We fixed a portion of the infected host tissue in Bouin's fluid and studied for any intra-cellular developmental stage of the parasites. We collected the cysts from infected hosts' hindgut and cultured them in a moist chamber for sporulation [5]. We took photomicrographs with an Olympus camera (Model C5060) attached to the same microscope. We took all the measurements in each case with calibrated ocular micrometers (μm). We present the minimum and maximum values, followed by the arithmetic mean, standard deviation, and sample size in parentheses. We followed the keys to the identification of species [6].

Results

Didymophyes bayaramae sp.nov.

Phylum : Apicomplexa Levine, 1988
Order : Eugregarinorida Léger, 1900
Family : Didymophyidae Léger, 1892.
Genus : Didymophyes Stein, 1848

Taxonomic Summary

Type host : *Aphis fabae*
Type locality : Imphal, Manipur, India.
Site of Infection : Mid-gut of the host.

Prevalence : 25 out of 60 (33.3%)

Holotype: With the No. M.U. / 01/14, we deposited the slide in the Museum of Parasitology Laboratory, Dept. of Zoology (Life Science), Manipur University, Canchipur.

Paratype: With the No. M.U./02-20/14, We deposited the slides in the museum of Parasitology Laboratory, Department of Life Sciences Manipur University, Canchipur, India

Etymology: We named the species after Prof. Bayram Göçmen, Protozoologist - Parasitology Research Laboratory, Zoology Section, Department of Biology, Faculty of Science, Ege University, Bornova, Izmir, Turkey, for his outstanding contribution to the field of Zoology.

Descriptions

Morphology of the trophozoite: The total body is Vase-like, and it's measuring 138.7 - 142.8µm (140.81 ± 1.22), epimeric papilla-like, becomes disc-like with prominent ridges radiating from the whole mouth, the top of the protomerite. Protomerite is somewhat conical in shape. Deutomerite is spherical, and its nucleus lies at its posterior portion. The shape of the nucleus is spherical to rounded and lies posterior to the deuterate. The nucleus has a distinct nuclear membrane and a large endoplasm. The cytoplasm of the deuterate is more granulated than the other body's.

Sporadic and association: Solitary as well as associative. Young sporadins are elongated, with hemispherical or egg-shaped protomerite, broader than long to ovoidal in shape. Deutomerite is elongated to obese in shape. The mature sporadic, however, are cylindrical to vase-like with ellipsoidal to rhomboidal protomerite and cylindrical deuterate.

During the association, we obtained large amounts of sporadic data. The association is always caudal-frontal. The associating partners exhibit bending maneuvers and become enclosed in the cyst wall. Occasionally, we observe the primate to retain the papilla-like epimerite during early association.

Gametocyst and Spore: Rounded gametocytes are blackish in color, single-walled, spherical bodies, measuring about 79.7 µm to 81.6 µm (80.69 ± 0.57) in dimension. After 48 hr of development under a moist chamber, the cysts die in simple rupture, liberating groups of spores or grapes-like structures. Spores are double-walled, smooth ellipsoidal measure 61.7µm to 63.7µm (65.8 ± 0.61). We provide all measurements and photographs in Table 1 and Figure 1.

Table 1. Measurements of 20 Specimens of *Didymophyes bayaramane* sp.nov. We present all measurements in µm.

S.N.	TL	LE	LP	LD	LN	WE	WP	WD	WN	L.P.:TL	W.P.: W.D.
1	138.70	15.8	61.14	79.77	13.44	13.9	36.33	69.47	-	1:2.2	1:1.91
2	138.85	16.9	61.15	79.85	13.55	14.3	36.45	69.58	-	1:2.23	1:1.31
3	138.95	17.6	61.21	79.99	13.67	15.1	36.55	69.67	-	1:2.27	1:1.90
4	139.10	17.8	61.22	80.0	13.89	15.3	36.66	69.78	-	1:2.28	1:1.90
5	139.97	-	61.25	80.15	13.94	-	36.77	69.89	-	1:2.28	1:1.90
6	140.11	-	61.35	80.25	14.0	-	36.84	70.0	-	1:2.29	1:1.90
7	140.25	-	61.49	80.34	14.12	-	36.95	70.15	-	1:2.3	1:1.89
8	140.85	-	61.55	80.44	14.23	-	37.0	70.27	-	1:2.4	1:1.89
9	140.25	-	61.75	80.56	14.34	-	37.11	70.38	-	1:2.5	1:1.90
10	140.85	-	61.95	80.67	14.34	-	37.25	70.49	-	1:2.6	1:1.89
11	140.95	-	62.11	80.74	14.45	-	37.31	70.57	-	1:2.7	1:1.88
12	141.10	-	62.35	80.85	14.56	-	37.44	70.64	-	1:2.8	1:1.88

13	141.15	-	62.75	80.99	14.67	-	37.57	70.78	-	1:2.2	1:1.89
14	141.35	-	62.85	81.0	14.75	-	37.68	70.84	-	1:2.2	1:1.88
15	141.45	-	62.99	81.13	14.89	-	37.74	70.99	-	1:2.2	1:1.89
16	141.55	-	63.25	81.27	14.99	-	37.82	71.0	-	1:2.2	1:1.88
17	141.75	-	63.35	81.34	15.0	-	37.99	71.15	-	1:2.2	1:1.87
18	141.85	-	63.45	81.44	15.1	-	38.0	71.21	-	1:2.2	1:1.89
19	141.99	-	63.65	81.55	15.2	-	38.11	71.34	-	1:2.2	1:1.87
20	142.8	-	63.75	81.66	15.3	-	38.25	71.44	-	1:22.2	1:1.86

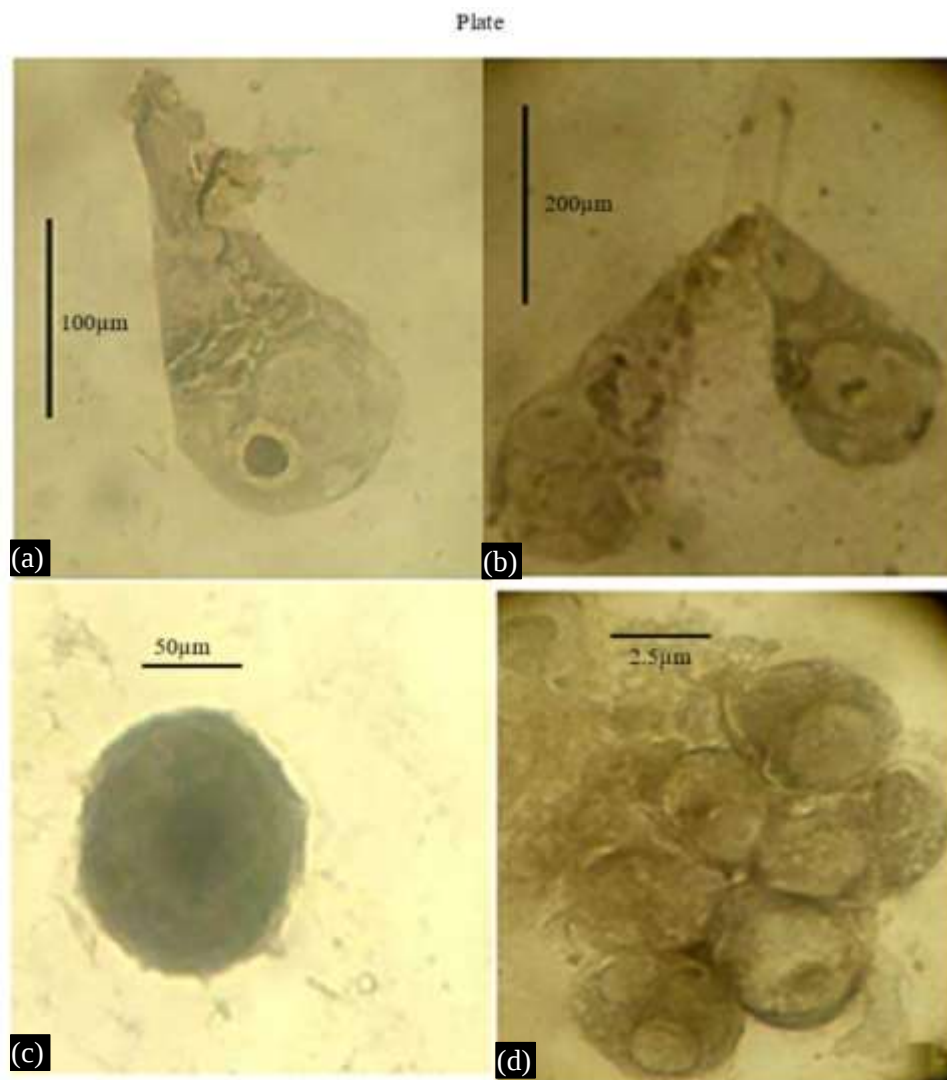


Figure 1. (a–d). Photomicrographs of Different Stages of Life Cycle of *Didymophyes bayaramae* sp. nov.; (a) Mature Trophozoite, (b) Syzygy, (c) Gametocyst, and (d) Spores.

DISCUSSION

Didymophyes [7] protozoan have pointed papilla-like epimeric, spherical cysts, dehiscence by simple rupture, ellipsoidal spores, and satellites without sputum [8]. The species under discussion shows significant morphological variabilities compared with other species of *Didymophyes*. We compared the present species with closely related species, *Didymophyes rigidus* [9] and *Didymophyes berhamporensis* [10].

The present species is similar to *Didymophyses rigidus* in that it has vase-like trophozoites. The epimerites' shape is identical in a papilla-like structure, which becomes disc-like with protomerite ridges. However, the ridges in *Didymophyses rigidus* radiate from the central area of protomerite, as observed from the top. But in the case of *Didymophyses Bayaramae* sp. nov., a prominent ridge radiates throughout the body of the protomerite as observed from the top. The two species differ in the shape and structure of different body parts. The protomerite of *Didymophyses rigidus* is rectangular or hemispherical and broader than long, while the protomerite of *Didymophyses bayaramae* sp. nov. is conical. The deutomerite of *Didymophyses bayaramae* sp. nov. is more or less spherical, and the nucleus lies at the posterior portion. In contrast, in the case of *Didymophyses rigidus*, the posterior end of the deuterate firmly fits in the cup-like depression at the anterior end of the satellite. The deutomerite of primate is round while that of the satellite is gradually narrower in shape. The nucleus of *Didymophyses bayaramae* sp. nov. is simply round and possesses a distinct nuclear membrane and a large endoplasm, while that of *Didymophyses rigidus* is ovoidal to elliptical. The sporadic in *Didymophyses rigidus* is solitary or associative and ovoidal to cylindrical are solitary or associative, elongated with hemispherical or egg-shaped protomerite, broader than long to ovoidal in shape, cylindrical to vase-like with ellipsoidal to rhomboidal protomerite and cylindrical. In the case of *Didymophyses bayaramae* sp. nov., sporadins are solitary or biassociative, elongated with hemispherical or egg-shaped protomerite, broader than long to ovoidal in shape, cylindrical to vase-like with ellipsoidal to rhomboidal protomerite and cylindrical deuterate. Gametocytes in *Didymophyses bayaramae* sp. nov. are round, while those of *Didymophyses rigidus* are spherical. Spores in *Didymophyses bayaramae* sp. nov. is spherical, while in *Didymophyses rigidus*, they are ellipsoidal.

The present species also differs from *Didymophyses berhamporensis* because it has different body parts shapes. The trophozoite is cylindrical in *Didymophyses berhamporensis* while it is vase-like in *Didymophyses bayaramae* sp. nov. Papilla is a straightforward structure in the case of *Didymophyses berhamporensis* while in the present species, the structure is with radiating ridges. Protomerites of *Didymophyses berhamporensis* are spatulate, while the present specimen has conical protomerite. Deutomerite of *Didymophyses bayaramae* sp. nov. is obese with a rounded posterior region, while in *Didymophyses berhamporensis*, it is spherical. The nucleus is rounded in *Didymophyses Jayaraman* sp. nov., while it is spherical in *Didymophyses berhamporensis*. Sporadin in *Didymophyses bayaramae* sp. nov. is hemispherical, while it is cylindrical in *Didymophyses berhamporensis*. Gametocytes are round in the present species, while *Didymophyses berhamporensis* is pitcher-shaped. Spores are spherical in the case of *Didymophyses bayaramae* sp. nov., while it is ellipsoidal in the case of *Didymophyses berhamporensis*. Table 2 shows the differences in the structural features.

Considering all aspects of differences, we believe and project the present specimen as a new species. Hence, we name the species as *Didymophyses Bayaramae* sp. nov.

Table 2. Differences in Characters of *Didymophyses bayaramae* sp. nov. with *Didymophyses rigidus* and *Didymophyses berhamporensis*.

Characters	<i>Didymophyses rigidus</i>	<i>Didymophyses berhamporensis</i>	<i>Didymophyses bayaramae</i> sp. nov.
Total Length	94.5 µm in length	48.6-54.0µm in length	142.8 µm in length
Trophozoite	Vase - like	Cylindrical	Vase -like
Epimerite	Papilla-like, it becomes disc-like with protomerite ridges radiating in the central area when viewed from the top.	Simple Papilla like	Epimerite becomes disc-like, with prominent ridges radiating throughout the body of protomerite as observed from the top.
Protomerite	Rectangular or hemispherical and is	Spatulate shape, separated from deuterate by a convex sputum	Protomerite is somewhat conical in shape

	broader than long		
Deutomerite	The posterior end firmly fits in the cup-like depression with the anterior satellite. The Deutomerite of the primate is round, while that of the satellite is gradually narrower in shape.	It is the largest segment, obese in shape with a round posterior extremity.	Deutomerite is spherical, and the nucleus lies at the posterior portion.
Nucleus	Ovoidal to elliptical	It is spherical; the pellicle is very thin, and epicedial striations are observable.	Elliptical with a distinct nuclear membrane and large endoplasm
Sporadic	Solitary or associative, Ovoidal to cylindrical	Cylindrical in shape, either solitary or associative	Solitary or associative; elongated, with hemispherical or egg-shaped protomerite, broader than long to ovoidal in shape. Cylindrical to vase-like with ellipsoidal to rhomboidal protomerite and cylindrical deuterate
Gametocyst	Spherical	Picher shaped	Rounded gametocytes are blackish
Spore	Ellipsoidal	Ellipsoidal	Spherical
L.P.: T.L.	1:8.02	-	1:2.24
W.P.: W.D.	1: 1.96	-	1: 1.86
Host	<i>Oryzaephilus mercator</i>	<i>Gymnopleurus parvus</i>	<i>Aphis fabae</i>
Locality	India	Berhampore, Murshidabad, West Bengal	Manipur, India

CONCLUSION

The newly identified septate gregarine species, *Didymophyses jayaraman* sp. nov., found in *Aphis fabae* from Imphal, Manipur, India, exhibits distinct morphological features differentiating it from closely related species. Its unique characteristics and high infection prevalence underscore its significant impact on the host's intestinal tissues.

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