# TWO NEW ENDOPARASITIC CEPHALINE OR SEPTATE GREGARNES (APICOMOPLEXA: PROTOZOA) FROM PADDY PESTS OF MURSHIDABAD DISTRICTS, WEST BENGAL, INDIA

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#### Abstract

This paper deals with the morphology and life history of two new species of septate gregarines (Apicomlexa: Sporozoea) under two different genera namely Stylocephatus Ellis, 1912 and Didymophyes Stein, 1848. These parasites are Stylocephatus janovyi sp. nov. from Scleron irregulate and Didymophyes berhamporensis sp. nov. from Gymnopleurus parvus (Macl.).

#### KEYWORDS

Apicomplexa, Didymophyes, Sporozoea, Stylocephalus

Two different species of cephaline gregarines (Apicomplexa: Sporozoea), one under the genus *Stylocephalus* Ellis, 1912 and the other under the genus *Didymophyes* Stein, 1848 were identified by us during our investigation of these parasites from field pests of Berhampore, Murshidabad, West Bengal, India. The parasites reported under two different genera *viz.*, *Stylocephalus* and *Didymophyes* widely differ from the ones described earlier under the same genera.

## MATERIALS AND METHODS

The host insects were collected from paddy fields and brought alive to the laboratory for investigations. The insects were decapitated, their gut carefully dissected out under a dissecting binocular and gently pressed for the parasites to come out from the gut lumen. Their smear preparations were fixed in Schaudinn's fluid and subsequently stained with Heidenhain's haematoxylin. Portions of midgut of infected host were fixed in Bouin's fluid and histological sections were studied for any intracellular developmental stage of the parasites. Cysts were collected from the hind gut of infected hosts and cultured in moist chamber for sporulation (Sprague, 1941).

Measurements of all figures in the text, unless otherwise mentioned, are in micrometers  $(\mu m)$ 

## STYLOCEPHALUS JANOVYI SP. NOV. Figs. 1(A-G)

## Material examined

<u>Type</u>: Berhampore, Murshidabad, West Bengal, India, ST/0010/06 deposited in the Museum of Department of Zoology, University of Kalyani, Kalyani. Host: *Scleron irregulate* 

#### Etymology

The specific epithet "janovyi" is given after the name of Prof. John Janovy, Jr. of University of Nebraska, Lincoln, USA, for his outstanding contribution in the field of picomplexan biology.

#### Observations

Development: Earliest developmental stage is intracellular with elongated body containing undifferentiated nucleus (Fig. 1A).

Trophozoite: Elongated trophozoites measuring 147.9 to 196.1 in total length. Short, tubular epimerite with expanded upper end bears a fine, long, pointed whip like structure and with hyaline cytoplasm, measuring 34.4-48.2 x 5.2-6.8 (Fig. 1B,C). The dome-shaped protomerite measures 20.6-27.5 in diameter and with uniform cytoplasmic granulation. Elongated, cylindrical deutomite, measuring 89.4-123.8 x 27.5-44.7, separated from protomerite by a distinct septum. Outer periphery of deutomerite is densely granulated while the central cytoplasmic portion is less coarse. The more or less elliptical nucleus with a distinct karyosome, is located anteriorly to the deutomerite and measures 12.0-27.5 x 6.8-11.0.

Sporadin and association: The elongated sporadins are solitary or biassociative and found in the mid gut lumen. Sporadins measures 96.3-189.2 in total length (Fig. 1D). Sub spherical protomerite, 17.2-27.5 x 20.6-30.9 in size, is separated from an elongated deutomerite by a prominent septum. Deutomerite measures 79.1-168.6 x 27.5-51.6. Ovoidal nucleus with two distinct karyosomes, measuring 12.0-17.2 x 6.8-13.8, located mostly at the anterior half of the deutomerite. Cytoplasmic granulation of protomerite and deutomerite is more or less uniform, although mature sporadins possess some less granulated areas. Pellicle is thin and epicyteal striations are not discernable. The characteristic frontal or head to head association of genus Stylocephalus is found in smear preparation (Fig. 1E).

Gametocyst and spore: Freshly collected gametocyst is spherical in shape and opaque white in colour. The cyst measures 412.4 x 396.5 in average dimensions (Fig. 1F). The outer margin of the cyst wall is finely papillated. The colour of the cyst turns brown after 48hr dehisces by simple rupture after 80hr of development inside the moist chamber. The hatshaped spores measuring 8.5 x 7.4 in diameter are liberated in long chains. The arrangement of spores is alternate. Spores are united sidewise by two black bulbous joints to form the chain (Fig. 1G).

Prevalence: 28 out of 35 (80%).

## Discussion

The species described here shares the common characters of the genus Stylocephalus in having a dilated papilla-like epimerite at the end of a long neck, hat-shaped spores and

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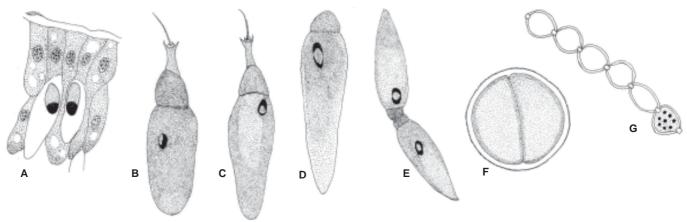


Figure 1. Camera lucida drawings of different life cycle stages of Stylocephalus janovyi sp. nov.

A - Early developmental stage; B &C - Elongated trophozoites; D - Elongated sporadin; E - Syzygy;

F - Gametocyst; G - Spores

cysts covered with minute papillae. It has also several short pointed epimerite, the distal portion of which is fine and characteristically whip-like and proximal portion short, tubular; very small size of the spore which are alternately arranged in the chain. The distal end of the deutomerite in the sporadin is somewhat pointed and bent on its axis. The gametocyst possess small protuberances at its outer margin which is also seen in case of S. bahli Mishra, 1941. But the structure of the epimerite of the present form has got no resemblance with that of S. bahli. Both solitary and frontal syzygy are found in case of S. bahli and the present form but frontal association is very rare in S. apapillatus Halder & Chakraborty, 1979. The host of both S. bahli and S. apapillatus is Gonocephalum sp. while the present form is found from an entirely different host Scleron irregulate. It is therefore, considered as new to science for which the name Stylocephalus janovyi is proposed.

## **DIDYMOPHYES BERHAMPORENSIS** SP. NOV. Figs. 2(A-F)

### Material examined

<u>Type:</u> Berhampore, Murshidabad, West Bengal, India, DP/0011/06 deposited in the Museum of Department of Zoology, University of Kalyani, Kalyani. Host: *Gymnopleurus parvus* (Macl.)

#### Etymology

The specific epithet "berhamporensis" is given after the name of place of collection of the host insects.

## Observations

Development: No intracellular developmental stages are formed.

Trophozoite: Freshly viewed trophozoites are solitary and opaque white in colour. The elongated cylindrical body of fully grown trophozoites measure 48.6-54.0 (average: 51.3) in total length (Figs. 2A,B). The simple papilla like epimerite with fine cytoplasmic granules measure 2.7-4.9 (average: 4.17)

in length and 3.24-5.94 (average: 4.41) in width. The spatula shaped protomerite with a concavity in its proximal part, measures 8.1-10.8 in length (average: 9.45) and 10.8-12.15 in width (average: 11.61). The protomerite is separated from the deutomerite by a convex septum. The largest segment deutomerite is obese in shape and its posterior extremity is rounded. It measures 34.3-40.5 in length (average: 37.53) and 10.8-17.82 in width (average: 14.94). The cytoplasmic granules of protomerite is very fine but that of deutomerite is uniformly coarse.

The nucleus is spherical measuring  $8.46 \times 9.99$  in diameter. The pellicle is very thin and epicyteal striations are not observable excepting in a slight portion of the protomerite.

Sporadin and association: The cylindrical sporadins are either solitary or biassociative measuring 37.8-64.8 in total length (average: 52.02) (Fig. 2C). The hemispherical protomerite is with greater width than that of its length and measures 11.63 x 14.76. The protomerite is separated from the deutomerite by a distinct concave septum. The cylindrical deutomerite is broadest at its distal portion and with a rounded base. It measures 28.35-49.95 in length (average: 37.53) and 13.5-33.5 in width (average: 14.94). The cytoplasm of both proto and deutomerite is uniformly granulated; the granules are fine in the protomerite and coarse in the deutomerite. The nucleus is more or less oval in shape with coarse chromatin granules distributed throughout the nucleoplasm. It measures 6.21-13.5 in length (average: 8.46) and 6.21-17.55 in width (average: 9.99). The nucleus is situated in the anterior half of the deutomerite. Caudo-frontal associations are always found in smear preparations (Fig. 2D). The protomerite of the primite is nearly dome shaped while the satellite is devoid of any protomerite. The posterior ends of the deutomerites of both primite and satellite are flat. The position of the nucleus in both the deutomerites is more or less similar.

Gametocyst and spore: The gametocysts are collected from the hind gut of the infected hosts and freshly collected ones are pitcher like and milky white in colour, measuring 125.7~x 84.8 in dimension (Fig. 2E). After about 22 hours of development inside the moist chamber the cyst dehisces by

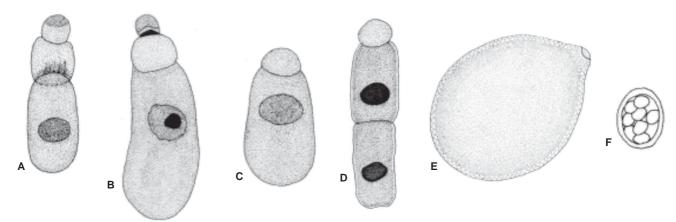


Figure 2. Camera lucida drawings of different life cycle stages of Didymophyes berhamporensis sp. nov.

A & B - Trophozoites; C - Sporadin; D - Syzygy; E - Gametocyst; F - Spore

simple rupture and the spores come out through a pore at one side. The ellipsoidal spores measuring  $7.8 \times 5.5$  in diameter are liberated in a mass (Fig. 2F). Formation of eight sporozoites is completed within 9 to 10 hours after sporulation.

Prevalence: 8 out of 37 (21.6%).

#### Discussion

In having sporadins in association and satellite without septum, the gregarine is at once placed in the genus *Didymophyes* Stein, 1848 under the family Didymophyidae Léger, 1892. It resembles *Didymophyes indiae* Kundu, 1980 regarding the structure of the nucleus and also in having a concavity in the proximal end of the protomerite of the trophozoite. So far protomerite shape is concerned it also shows resemblance with *D. minuta* (Ishii) Watson, 1916 and *D. paradoxa* Stein, 1848. The present species, however, differs from these three species in all other aspects like shape of the deutomerite, gametocyst and spores. In the ratios of LP and TL and WP and WD it also shows affinity with *D. leuckarti* Marshall, 1893.

The present species is most peculiar in having pitcher like gametocysts and ellipsoidal spore. It is therefore, considered as new to science for which the name *Didymophyes berhamporensis* sp. nov. is proposed.

### Conclusion

Like majority of cases, the systematics of the two new species of septate gregarines described here have been determined by certain parameters like measurements of trophozoites or gammonts, their ratios as well as type of hosts.

In our earlier communication (Sengupta & Haldar, 1996) we have showed the relationships between total length and other trophozoite measurements in terms of regression lines and correlation coefficients and also established that although measurements are positively and significantly correlated with total length, the different correlation coefficients indicate that most increases in size with age are because of the lengthening of the deutomerite.

So far histological observations are concerned *Didymophyes* berhamporensis sp. nov. showed no tissue phases but the sections of host's gut in case of *Stylocephalus janovyi* sp. nov. reveal

certain features due to invasion of the parasite. The nuclei of the infected cells are displaced and increased basophilia is observed, thereby indicating some possibilities of the changes in the secretory habits of the cells. However, it is not known whether the infected cells recover these damages after the release of the intracellular parasites into the host gut lumen. Further research is therefore needed to have some clear idea about the histopathological damage of the insect hosts due to gregarine infection which can throw some light in the possible role of gregarines on the biological control of insect pests.

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