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STUDIES IN SEPTATE GREGARINES (APICOMPLEXA : SPOROZOEA) FROM INSECTS OF WEST BENGAL : SIX NEW SPECIES OF HIRMOCYSTIS, GREGARINA, STENOPHORA AND HYALOSPORINA

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INTRODUCTION

During the course of our investigation on the protozoan parasites of insects we have obtained six new species of septate gregarines (Apicomplexa : Sporozoea) belonging to the genera *Hirmocystis, Gregarina, Stenophora and Hyalosporina.* The structures, life histories and affinities of these gregarines have been elaborately described in this communication. It may be mentioned here that the genera *Gregarina. Hirmocystis, Stenophora* and *Hyalosporina* were established by Dufour (1828), Labbe (1899), Labbe (1899) and Chakravarty (1935) respectively. Subsequently, many new species were added to all these genera. Haldar et al. (1985a, b) compiled a list of Indian species from various arthropods.

MATERIALS AND METHODS

The different insect hosts were collected from various places in West Bengal (TABLE 1) and were brought alive to the laboratory for microscopic examination of protozoan parasites. These were dissected out, smeared on slides, fixed and stained by the usual Heidenhain's haematoxylin method. Development of gregarines was studied by staining sections of host gut. For details, please see Ghose et al. (1986). The type materials are deposited in the National Collection, Z.S.I. (Calcutta).

OBSERVATIONS

Genus **Hirmocystis** Labbe, 1899

Sporadins in association of 2 to 12 ; epimerite conical or cylindrical knob ; cysts spherical and ovoidal spores.

Hirmocystis alphitobiusi n. sp.

(Text Figs. 1-5)

Host : *Alphitobius piceus* Oliver (Coleoptera : Tenebrionidae).

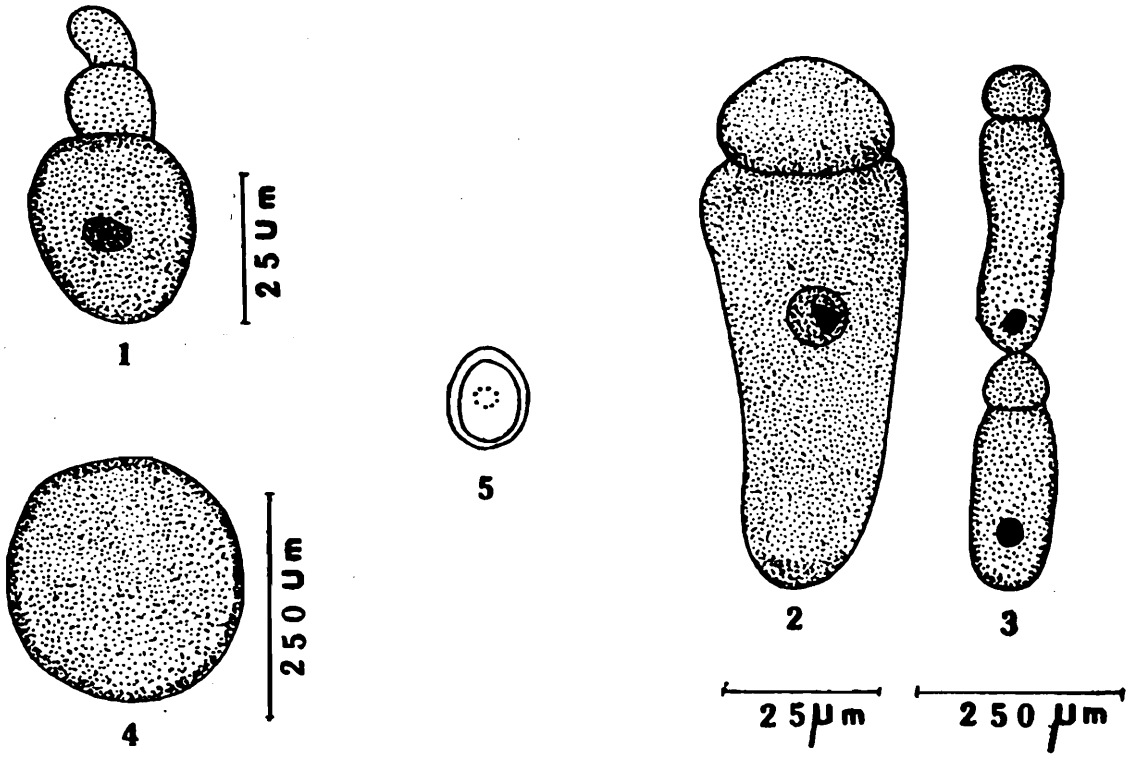
Development : Extracellular.

TABLE I

Showing names of the hosts, names of the parasites, places of collection, number of hosts examined, number of hosts parasitized and percentages of infection of six new species of gregarines from insects.

Sl. No.	Name of the hosts	Name of the parasites	Place of collec-collection	No. of hosts examined	No. of hosts parasitized	% of infection
1.	*Alphitobius piceus* Oliver	*Hirmocystis alphitobiusi*	Naihati, W.B., India	75	55	73.3
2.	*Coccinella transversalis* (Fabr.)	*Hirmocystis coccinelli*	Kalyani, W.B., India	500	75	15.0
3.	Larva of *Triboliu.n Castaneum* (Herbst)	*Gregarina amojii*	Chandannagar, W.B., India	191	37	17.3
4.	*Verania discolor* (Fabr.)	*Gregarina veraniae*	Kalyani, W.B., India	65	19	29.2
5.	An unidentified harpagophorid Millipede	*Stenophora harpagophora*	Kalyani, W.B., India	50	40	80.0
6.	An unidentified harpagophorid Millipede	*Hyalasporina mukundi*	Kalyani, W.B., India	40	30	75.0

Trophozoite : Solitary, elongated ; knob-like epimerite and ovoidal protomerite. The deutomerite is cylindro-conical in shape with rounded end. Spherical nucleus situated at the centre of the deutomerite.



Text Figs. 1-5. Camera lucida drawings of different life-cycle stages of *Hirmocystis alphitobiusi n. sp.* — 1. Fully grown trophozoite ; 2. Sporadin ; 3. Association ; 4. Gametocyst ; 5. Spore.

Sporadin and association : Cylindro-conical in shape with hemispherical protomerite which is broader than long. Elongated cylindrical deutomerite broadest at the protomerite-deutomerite junction with a rounded posterior end. Cytoplasm is densely granulated in protomerite than the deutomerite. Epicyteal striations are absent. Spherical nucleus with an endosome present centrally.

Syzygy caudo-frontal. Primite is larger than satellite.

Gametocyst and spore : Gametocyst spherical in shape measuring 133.3 μm in diameter. After 120 hrs. cyst dehisces by simple rupture liberating double walled, oval spore. Each spore measures 5.0 μm × 3.3 μm. Formation of eight sporozoites is completed after 30 hrs. of development.

Measurements (in microns) :

Trophozoite

TL = 50.0-66.6 (55.5) ;
LE = 8.3 (8.3) ; WE = 8.3 (8.3) ;
LP = 16.7 (16.7) ; WP = 8.3-16.7 (11.1) ;
LD = 25.0-41.7 (30.6) ; WD = 8.3-16.7 (11.1) ;
LN = 8.3 (8.3) ; WN = 8.3 (8.3) ;

Sporadin

TL = 58.3-166.6 (118.0) ; WP = 8.3-50.0 (33.2) ;
LP = 8.3- 41.7 (21.6) ; WD = 8.3-58.3 (37.7) ;
LD = 42.7-141.6 (98.6) ; WN = 8.3-16.7 (9.3) ;
LN = 8.3-25.0 (10.8) ;
LP:TL = 1:3.0-16.0 (6.13) ;
WP:WD = 1:0.04-1.5 (0.92).

Material : Holotype ; Coll. No. X 6/1 S.C. Datta Coll. dated 9.10.1979. Type locality ; Naihati, W.B., Paratype ; Coll No. X 6/2 and 3.

Affinities : The gregarine possesses close resemblances with *H. ovalis* Crawley, 1903 and *H. bengalensis* haldar and Chakraborty, 1979 in the LP : TL and WP : WD values. But in *H. ovalis* the cyst and spores are unknown while in *H. bengalensis* the development is intracellular and spores possess four knob-like projections which are lacking here. In view of these differences the parasite is assigned a new taxonomic status and is named *Hirmocystis alphitobiusi* n.sp., after the generic name of its host.

Hirmocystis coccinelli n. sp.
(Test Figs. 6-11)

Host : Coccinella transversalis (Fabr.) (Coleoptera : Coccinellidae)

Development : Extracellular.

Trophozoite : Solitary, opaque white in colour ; simple hyaline papilla-like epimerite. The protomerite is hemispherical and the deutomerite is spherical in shape. Nucleus is oval with distinct nuclear membrane.

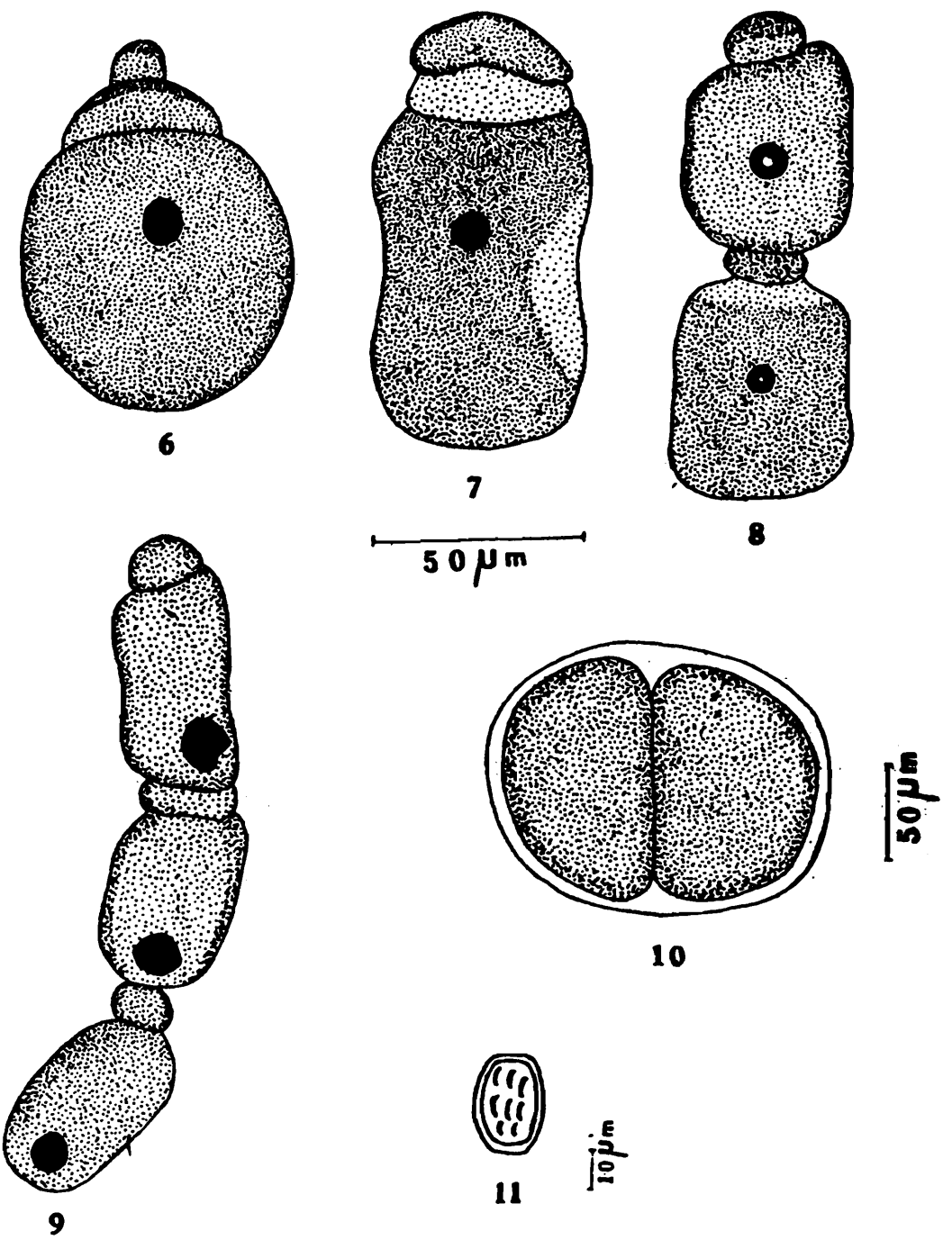
Sporadin and association : Barrel-shaped sporadin. Protomerite is hemispherical

with a convex free end and the deutomerite is cylindrical with a rounded posterior end. Cytoplasm is uniformly granulated. The pellicle is thin and epicyteal striations are not observable.

Caudo-frontal syzygy, 2-4 in number ; primite is larger than the satellite.

Gametocyst and spore : Gametocyst spherical measuring 150.0 μm — 155.0 μm×130.0 μm—140.0 μm. Cyst wall is thin and transparent enclosing two unequal gametocytes. After 24 hrs. of development the partition wall between the gametocytes disappears. The cyst dehisces by simple rupture after 120 hrs. of development.

Barrel-shaped, double-walled spores measure 10.0 μm × 5.0 μm with eight sickle-shaped sporozoites arranged irregularly in each spore.



Text Figs. 6-11. Camera lucida drawings of different stages of life-cycle of *Hirmocystis coccinelli* n.sp.—6. Trophozoite ; 7. Sporadin ; 8 and 9. Associations ; 10. Gametocyst ; 11. Spore.

Measurements (in microns) :

'Trophozoite

TL = 31.9-38.3 (35.1) ;
LE = 4.3-10.6 (7.5) ; WE = 6.4-8.5 (7.4) ;
LP = 6.4 (6.4) ; WP = 8.5-12.8 (10.7) ;
LD = 21.3 (21.3) ; WD = 12.8-21.3 (17.1) ;
LN = 4.3-6.4 (5.4) ; WN = 4.3.(4.3) ;

Sporadin

TL = 42.5-110.5 (73.9) ;
LP = 8.5- 17.0 (13.0) ; WP = 17.0-55.3 (23.4) ;
LD = 34.0-97.8 (60.9) ; WD = 25.5-76.5 (43.0) ;
LN = 4.3- 6.4 (5.8) ; WN = '4.3- 6.4 (5.8) ;
LP:TL = 1:3.0-12.5 (6.0) ;
WP:WD = 1:0.3-0.8 (0.6).

Material : Holotype : Coll. No. C_5 S.C. datta. Coll. dated 1.3.1979. Type locality; Kalyani University campus, West Bengal. Paratype ; Coll. No. C_6 and C_7.

Affinities : Possession of simple-papilla like epimerite, association of two's to four's and simple dehiscence of cyst place the parasite under the genus *Hirmocystis* Labbé. The gregarine bears close affinity with *H. dharwarensis* Uttangi and Desai, 1962 in LP:TL value but differs in all other respects. The parasite also shows some similarities with *H. alphitobiusi* n. sp. in the development, shape of the gametocyst and WP:WD values but differs in the shape of epimerite, protomerite, deutomerite, spore and LP:TL values. As such, the gregarine is designated as a new species and named *Hirmocystis coccinelli* n.sp. after the generic name of its host.

Genus **Gregarina** Dufour, 1828

Biassociative, Epimerite small, globular or cylindrical spores dolioform to cylindrical. Cyst dehisces by sporoduets.

Gregarina amojii n. sp.
(Text Figs. 12-17)

Host : Larva of *Tribolium castaneum* (Herbst) (Coleoptera : Curculionidae).

Development : Intracellular.

Trophozoite : Small globular or cylindrical. The epimerite is globular or knob-like with very less amount of cytoplasm. The protomerite is hemispherical, bell-shaped or dome-shaped. A concave septum separates the epimerite from the protomerite. The deutomerite is also variously shaped e.g., conical, cylindrical or globular. A rounded or spherical nucleus with nuclear membrane and an endosome is present at the posterior portion of the deutomerite.

Sporadin and association : Sporadins are solitary as well as biassociative. Globular to cylindrical in shape with subspherical or flat protomerite and obese to elongated deutomerite. The pellicle is thick and cytoplasm is finely granulated.

Caudo-frontal association is very firm. The protomerite of the primite is egg-shaped while that of the satellite is more or less rectangular.

Gametocyst and spore : Gametocysts are egg shaped, double-walled with a gelatinous outer membrane. The cyst measures 69.7 μm × 54.0 μm in diameter. After 72 hrs. of development six sporoducts are developed from the cyst surface. Eight hrs after the formation of sporoducts the spores are released in short chains.

Ellipsoidal spores measure 4.4 μm × 2.3 μm. Each spore contains eight pea-shaped sporozoites arranged irregularly.

Measurements (in microns) :

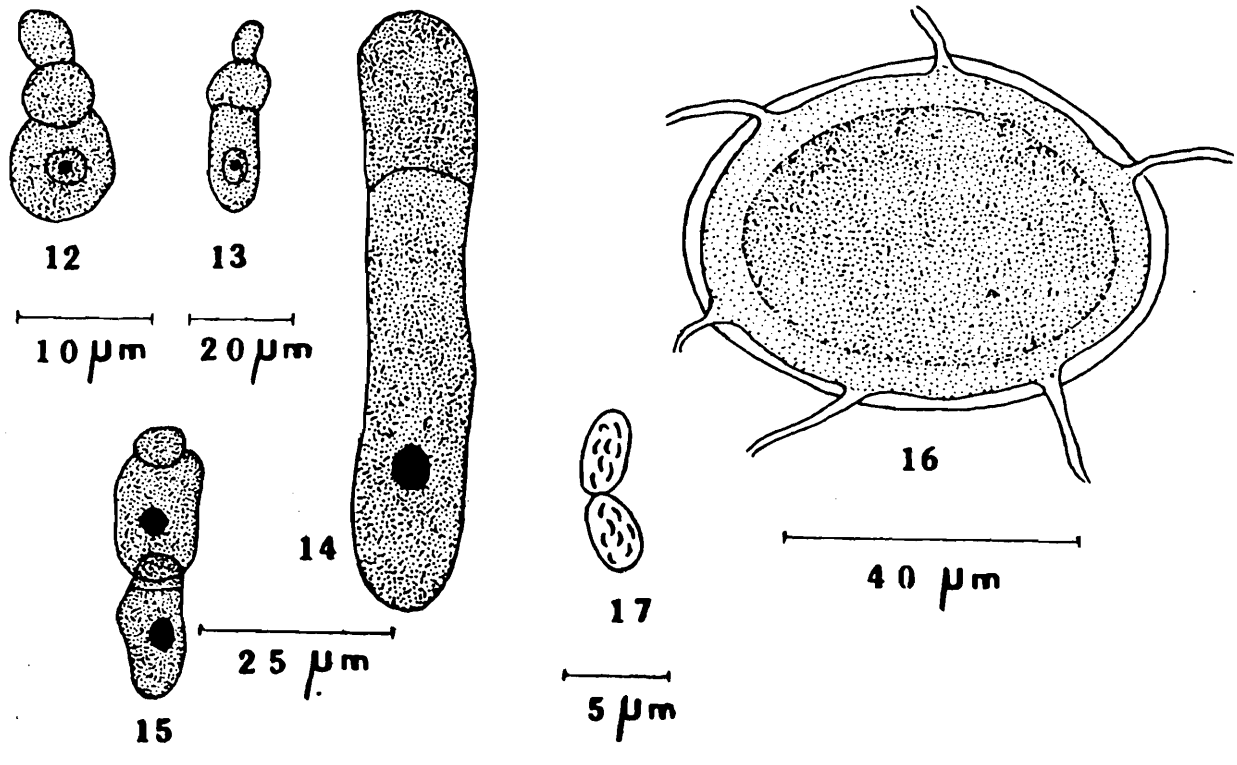
Trophozoite

TL = 18.36-31.05 (26.1) ;
LE = 5.4- 6.75 (5.7) ; WE = 5.4 - 7.0 (5.7) ;
LP = 5.4 - 8.1 (6.8) ; WP = 8.1 - 9.45 (8.7) ;
LD = 6.48-17.55 (13.4) ; WD = 8.1-13.0 (10.8) ;
LN = 3.0 - 4.85 (3.8) ; WN = 3.24- 5.4 (4.1) ;

Sporadin

TL = 17.55-68.04 (42.5) ;
LP = 6.75-18.9 (13.3) ; WP = 7.57-18.9 (13.4) ;
LD = 7.45-53.46 (28.3) ; WD = 8.1 -22.95 (14.1) ;
LN = 3.24-6.75 (5.5) ; WN = 4.59-6.75 (5.4) ;

LP:TL = 1:2.2-5.2 (3.3) ;
WP:WD = 1:0.7-1.5 (1.0).



Text Figs. 12-17. Camera lucida drawings of different stages of life-cycle of *Gregarina a.nojii* n. sp. — 12 and 13. Trophozoites ; 14. Sporadin ; 15. Syzygy ; 16. Gametocyst with six sporoducts 17. Spores ;

Material : Holotype ; Coll. No. BLT/$_4$ S Ghose Coll. dated 2.4.1984. Type locality ; Chandonnagore, W.B., Paratype ; Coll. No. BLT/$_5$ and BLT/$_6$.

Affinities : Four species of the genus *Gregarina* have so far been recorded from *Tribolium* spp. (Sokoloff, 1972 ; Ghose et al., 1986). These are *G minuta, G. cuneata, G. crassa* and *G. basiconstrictonea.* The species under report shows close similarities with *G. cuneata* Stein, 1848 in LP:TL and WP:WD ratios and formation of sporoducts but differs from it in the shape of epimerite, sporadin, gametocyst and spore. The gregarine exhibits close resemblances in the shape of association with *G. minuta* Ishii, 1914 but differs from it in all other characters. The gregarine also resembles *G. basiconstrictonea* in the general shape of the sporadin and WP:WD values, but markedly differs from it in other respects. The gregarine possesses characters like knob-like or globular epimerite and egg-shaped gametocyst, dehisces through six sporoducts liberating ellipsoidal spores. These characters are sufficient to erect a new species for it and the name *Gregarina amojii* n. sp. is proposed, after the Indian gregarinologist, Dr. S.D. Amoji of Gulbarga.

Gregarina veraniae n. sp.
(Test Figs. 18-22)

Host : Verania discolour (Fabr.) (Coleoptera ; Coccinellidae).

Development : Extracellular.

Trophozoite : Elongated ; knob-like epimerite, hemispherical protomerite and cylindrical deutomerite. Cytoplasm is uniformly granulated and epicyteal striations are not discernable.

Sporadin and association : Sporadins are elongated, cylindrical with dome-shaped protomerite and ovoidal or barrel-shaped deutomerite. A straight septum separates the protomerite from the deutomerite. Spherical nucleus is present posteriorly in the deutomerite.

Association is caudo-frontal ; protomerite of the satellite becomes concave for receiving the primite and thus reveals firm attachment between the two.

Gametocyst and spore : Gametocyst spherical, measuring 91.6 um×108.3 um in diameter. After 24 hrs. of development four sporoducts appear from the cyst surface. Each duct measures 41.7 um in length. At about 48 hours of development spores are liberated in chains through these sporoducts.

The spores are ovoidal measuring 8.3 um×4.2 um with eight sporozoites arranged in a circular fashion in each spore. Formation of sporozoites is completed at 48 hrs. of development.

Measurements (in microns) :

Trophozoite

TL = 40.7-51.0 (45.9) ;
LE = 1.7- 8.5 (5.1) ; WE = 1.7- 8.5 (5.1) ;
LP = 8.3- 8.5 (8.4) ; WP = 10.0-12.8 (11.4) ;
LD = 32.4-34.0 (33.2) ; WD = 16.6-17.0 (16.8) ;
LN = 4.2- 6.0 (5.2) ; WN = 4.0- 6.0 (5.2) ;

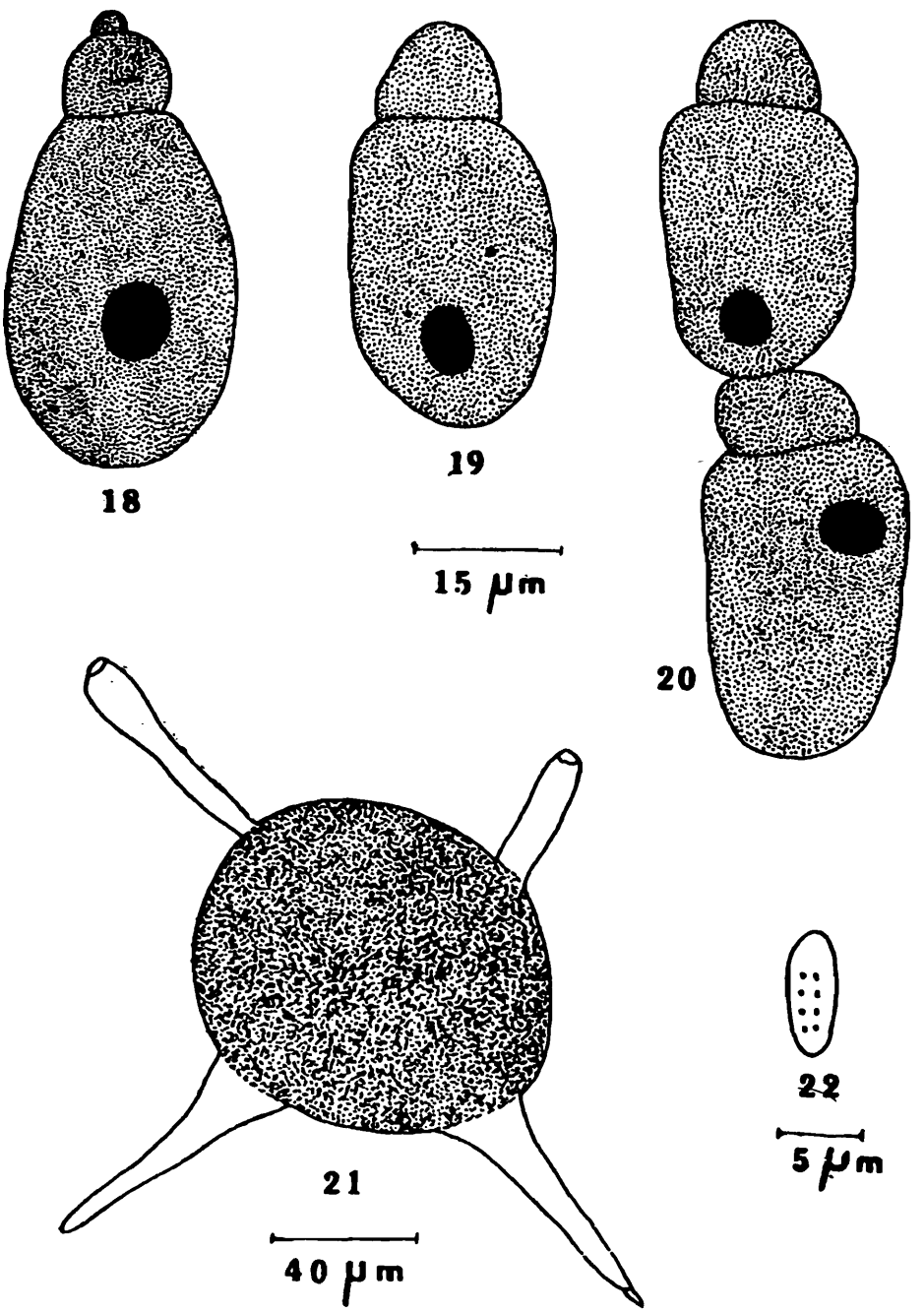
Sporadin

TL = 29.8-82.9 (48.9) ;
LP = 4.3-17.0 (8.5) ; WP = 8.5-21.3 (13.3) ;
LD = 25.5-65.9 (25.6) ; WD = 14.9-46.8 (23.1) ;
LN = 4.3- 6.4 (5.8) ; WN = 4.3- 6.4 (5.8) ;

LP:TL = 1:5.6 ;
WP:WD = 1:1.6.

Material : Holotype ; Coll. No. X5/1 S.C. Datta Coll. dated 8.10.1979. Type locality ; Kalyani University campus, W.B., paratype ; Coll. No. X5/3 and others.

Affinities : Presence of knob-like epimerite, biassociative sporadins and dehiscence through sporoducts are sufficient to place this parasite under the genus *Gregarina*.



Text Figs. 18-22. Camera lucida drawings of different stages of life-cycle of *Gregarina veraniae* n. sp. — 18. Trophozoite ; 19. Sporadin ; 20. Association ; 21. Gametocyst with four sporoducts ; 22. Spore.

The under report gregarine possesses close similarities in the shape of the epimerite with *G. alcidesii* Halder and Chakraborty, 1978 and *G. spraguei* Haldar and Chakraborty, 1978 ; however, it differs from these two species in other respects. Its ovidal spores and the ratios of LP:TL and WP:WD are distinctly separate from previously described species of the genus *Gregarina*. A new species *Gregarina veraniae* n.sp. is proposed here to accommodate the gregarine.

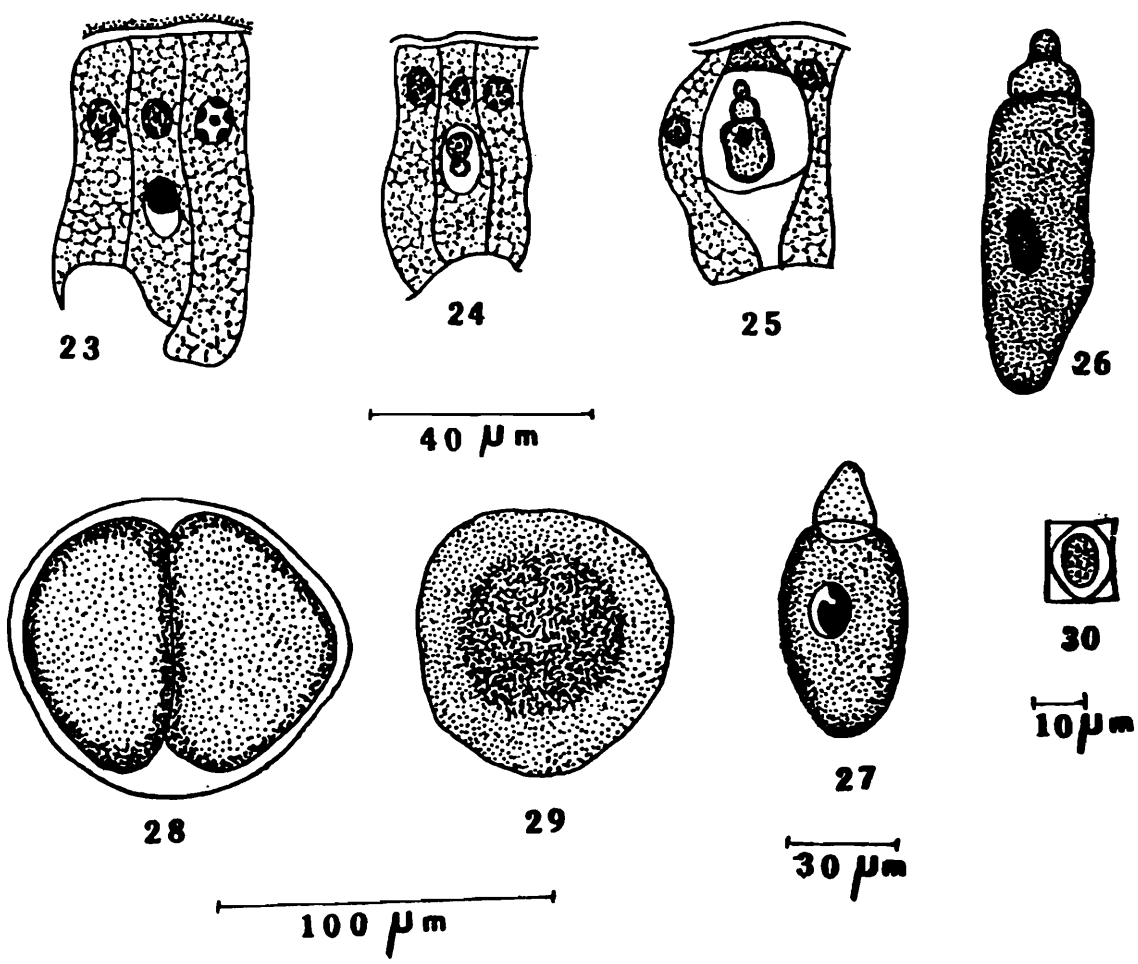
Genus **Stenophora** Labbe, 1899

Intracellular development. Sporonts solitary. Epimerite absent or simple. Cysts dehisce by simple rupture. Spores ovoidal with equatorial line, not extruded in chains.

Stenophora harpagophora n. sp.
(Text Figs. 23-30)

Host : An unidentified millipede (Spirosteptida : Harpagophoridae)

Development : Intracellular.



Text figs. 23-30. Camera lucida drawings of different stages of life-cycle of *Stenophora harpagophora* n. sp. — 23. Section of the host midgut showing the first intracellular stage of development of the parasite ; 24. Second intracellular stage of development of the parasite ; 25. A fully grown trophozoite within the epithelial cell of the midgut of the host ; 26. Trophozoite ; 27. Sporadin ; 28. An early gametocyst ; 29. Gametocyst before dehiscence ; 30. A spore with rectangular epispore ;

Trophozoite : Elongated. Epimerite is small, hyaline papilla like. The protomerite is dome-shaped while deutomerite is elongated with rounded end. The elliptical nucleus is situated anywhere in the deutomerite.

Sporadin : Solitary, cylindro-conical in shape. The protomerite is hemispherical and tapering towards the distal end. Cylindro-conical deutomerite with moderate amount of cytoplasm while the protomerite is almost free of granules. Nucleus with distinct nuclear membrane and a deeply stained endosome located at any place in the deutomerite.

Gametocyst and spore : Gametocyst rounded in shape measuring 133.3 um in diameter. The gametocytes are equal in size. After 144 hrs. of development the gametocyst dehisces by simple rupture releasing the spores in mass.

Ovoidal spores with two walls surrounded by a rectangular coat. Tuzet, Manier and Jolivet (1957) have called this as 'epispore' Each spore measures 4.4 μm × 3.9 μm. 24 hrs. after the dehiscene of cyst, sporozoites are formed and are arranged irregularly.

Measurements (In microns) :

Trophozoite

TL = 85.9-110.8 (94.7) ;
LE = 2.8 (2.8) ; WE = 2.8- 5.5 (3.3) ;
LP = 11.1- 13.9 (11.7) ; WP = 16.4-19.4 (17.2) ;
LD = 69.3- 83.1 (74.8) ; WD = 24.9-30.5 (27.1) ;
LN = 11.9- 16.6 (14.6) ; WN = 2.8- 5.5 (4.96) ;

Sporadin

TL = 69.7-152.4 (93.8) ;
LP = 8.3- 19.4 (13.6) ; WP = 13.9-27.7 (17.5) ;
LD = 58.2-135.7 (81.4) ; WD = 22.2—55.4 (28.4) ;
LN = 8.3—19.4 (12.4) ; LP:TL = 1:5.7-10.0 (7.3)
WN = 5.5 (5.5) ; WP:WD = 1:1.3- 2.0 (1.7)

Material : Holotype ; Coll. No. M 6/1 S.C. Datta Coll. dated 3.4.1980. Type locality ; Kalyani, W.B., Paratype ; Coll. No. M 6/2 and others.

Affinities : Possession of solitary sporadin, dehiscence of cyst by simple rupture, spore possessing an epispore and intracellular development are sufficient for the inclusion of the gregarine under the genus *Stenophora* Labbe. The parasite bears similarities in shape of protomerite and cyst with *S. conjugata* Rodgi and Ball, 1961 and *S. haplothysani* Tuzet, Manier and Jolivet, 1957 but differs in all other respect. It has also some resemblances with *S. mahabaleshwari* Amoji and Rodgi, 1972 and *S. haplothysani* Tuzet, Manier and Jolivet, 1957 in the shape of nucleus while it differs in all other characters. Therefore, the parasite described here as a new species, for which the name *Stenophora harpagophora* n. sp. is proposed after the name of the family to which the millipede belongs.

Genus **Hyalasporina** Chakravarty, 1935

Sporonts solitary ; epimerite small, tongue-like ; anisogametes ; cysts with

ducts ; spores oval with a hyaline membrane.

Hyalosporina mukundi n. sp.
(Text Figs. 31-36)

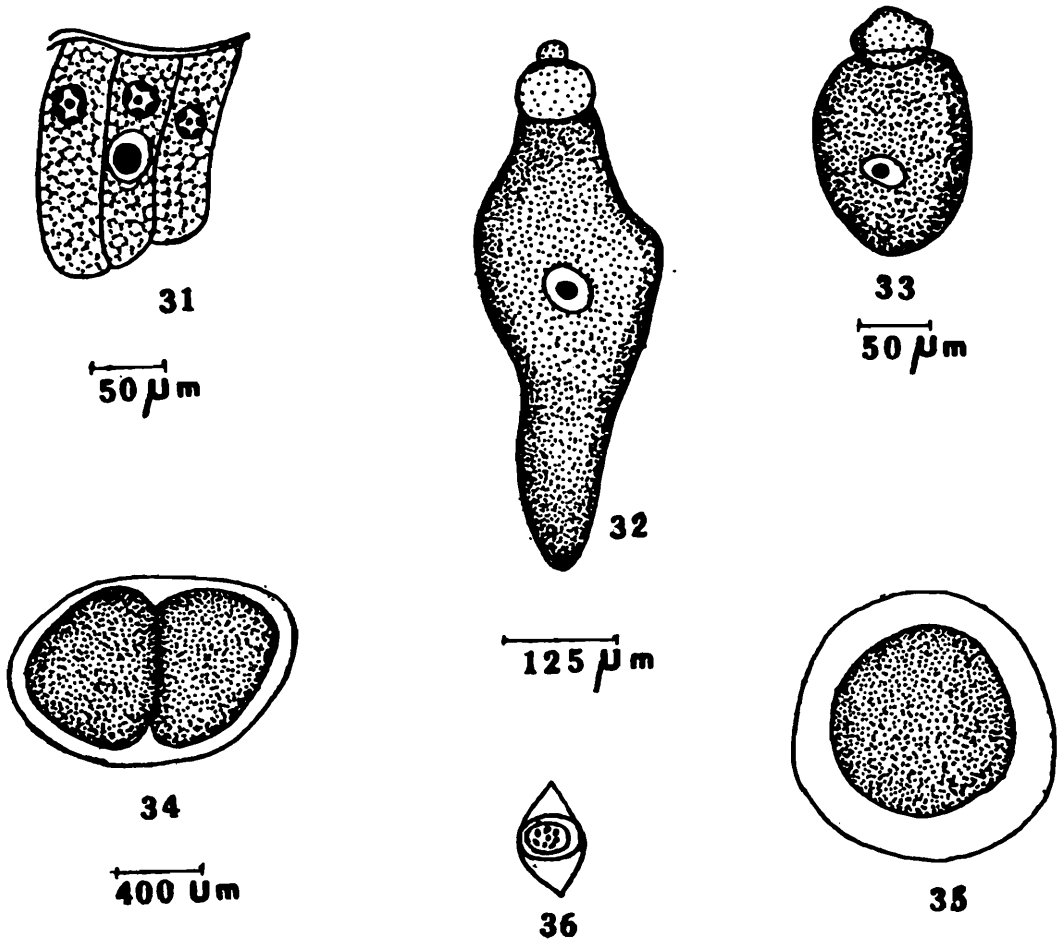
Host : An unidentified millipede (Spirosteptida : Harpagophoridae)

Development : Intracellular.

Trophozoite : Elongated with tongue-like epimerite which is always broader than long. The protomerite is hemispherical while the deutomerite is saucer-shaped. Cytoplasm is uniformly granulated. Spherical nucleus with distinct nuclear membrane.

Sporadin : Cylindro-conical with ovodial protomerite and conical deutomerite. Spherical nucleus. Cytoplasm is uniformly granulated.

Gametocyst and spore : Gametocyst opaque-white in colour, spherical in shape measuring 312.0 um in diameter. The gametocytes are equal in size. An ectocyst is present encircling the cyst measuring 62.4 um. After 216 hrs. the cyst dehisces by simple rupture liberating oval spore with two coats. A hyaline spindle-shaped membrane encircles the spores. Each spore measures 7.8 μm × 5.0 μm.



Text Figs. 31-36. Camera lucida drawings of different stages of life-cycle of *Hyalosporina mukundi* n. sp. — 31. Earliest intracellular stage with the epithelial cell of the host proventriculus; 32. Trophozoite ; 33. Sporadin ; 34. Early gametocyst ; 35. Gametocyst ; advanced stage ; 36. Spare with a hyaline coat.

Formation of sporozoites is completed after 24 hrs. of dehiscence whice lie scattered irregularly within the spore.

Measurements (in microns) :

Trophozoite

TL = 299.9 ;
LE = 8.3 ; WE = 16.7 ;
LP = 16.7 ; WP = 33.3 ;
LD = 274.9 ; WD = 83.3 ;
LN = 25.0 ; WN = 8.3 ;

Sporadin

TL = 83.3-266.6 (193.7) ;
LP = 16.7- 33.3 (19.6) ; WP = 16.7- 50.0 (27.9) ;
LD = 66.6-249.9 (165.8) ; WD = 33.3-133.3 (90.6) ;
LN = 16.7- 25.0 (20.8) ; WN = 16.7-25.0 (17.2) ;
LP:TL = 1:5.0 -14.0 (10.8) ;
WP:WD = 1:0.16- 0.75 (0.3).

Material : Holotype ; Coll. No. M7/1 S. C. Datta Coll. dated 8.10.1979. Type locality ; Kalyani University campus. Paratype ; Coll No. M7/3 and others.

Affinities : In having tongue-shaped epimerite, solitary sporadin, dehiscence of cyst by simple rupture, spores with hyaline membranes. The parasite satisfies its inclusion in the genus *Hyalosporina* Chakravarty, 1935. The presently described species has some resemblances with *H. Cambolopsisae* Chakravarty, 1935 in having tongue-like epimerite and ovoidal spores, but differs from the latter in other features. The parasite under report also shows some relationships with *H. rayi* Chakravarty, 1936 in having spherical gametocyst and general elongated shaps of the body but differs in all other characters. Thus in having some distinct features of its own the parasite designated as a new species and named *Hyalosporina mukundi* n. sp. after the Late Dr. Mukunda Murari Chakravarty who established the genus.

SUMMARY

The present paper deals with six new species of septate gregarines (Apicomplexa : Sporozoea) from insects.

REFERENCES

Chakravarty, M.M. 1935. Studies on sporozoa from Indian millipedes. IV. Life history of cephaline gregarine *Hylosporina cambolopsisae* n. gen., n. sp., Arch. protistenk., **86**:211-218.

Dufour, L. 1828. Note sur la gregarine nouveau genre de ver qui vit en tropeau dans les intestines de elivers insects. Ann. Sci. Nat., **13**(1):366-368.

Ghose, S., Sengupta, T and Haldar, D.P. 1986. Two new septate gregarines (Apicomplexa : Sporozoea), *Gregarina basiconstrictonea* n. sp. and *Hirmocystis*

oxeata n. sp. from *Tribolium castaneum* (Herbst). Acta Protozool., **25**(1):93-108.

Haldar, D.P., Sengupta, T and Ghose, S. 1985a. An annotated list of septate gregarines (Apicomplexa : Sporozoea) from Insects in India. Bull. Zool. Surv. India., 7(2-3):311-322.

Haldar, D.P., Sengupta, T and Ghose, S. 1985b. An annonated list of septate gregarines (Apicomplexa : Sporozoea) from Indian Arthropods. J. Beng. Nat. Hist. Soc., **4**(1):20-39.

Labbe', A. 1899. Sporozoa, In ' ' Das Tierreich" Friedlander. Berlin Leif, 5(20) : 1-180.

Sokoloff, A. 1972. The biology of Tribolium. Oxford Univ. Press. 1 and 2:249-294.