

## NOTES ON THE GREGARINES IN JAPAN, 1.

*Gregarina ampullaria* n. sp. from *Altica caerulescens* Baly and two other already known gregarines from Chrysomelidae.

By

Hyoma HOSHIDE and Kazumi HOSHIDE\*

(Received September 28, 1968)

Some beetles belonging to the family Chrysomelidae were examined mainly at Yamaguchi City and three species of cephaline gregarines - *Gregarina ampullaria* n. sp., *Gregarina munieri* (Schneider), *Gregarina crenata* (Bhatia and Setna) - were perceived as their parasites.

*Gregarina munieri* and *G. crenata* being reported previously by the senior author (Hyoma Hoshide) and reported again this time by the authors in cooperation. About these gregarines, a brief diagnostic description is given. With regard *Gregarina ampullaria* n. sp., they have studied its morphological and ecological characteristics and propose to put it between *G. munieri* and *G. crenata* as a new species.

In this paper, the following abbreviations were used.

TL=Total length of sporadin (or primite).	tl =Total length of satellite.
LP=Length of primite protomerite.	lp =Length of satellite protomerite.
WP=Width of primite protomerite.	wp=Width of satellite protomerite.
WD=Width of primite deutomerite.	wd=Width of satellite deutomerite.

### OBSERVATION

#### 1. *Gregarina munieri* (Schneider).

Hosts: *Cassida japana* Baly. Habitat: Intestine. Locality: Yamaguchi City.

Sporadins. (Fig. 1)

Biassociative, ellipsoidal to ovoidal. Primite protomerite subspherical, well rounded anteriorly, width is 1.2 to 1.5 times greater than height, sometimes a very shallow constriction at its anterior one-third. Satellite protomerite flattened, width is 2 times as large as height. Slight but conspicuous constriction at septum.

---

\* Zoological Institute, Faculty of Science, Hokkaido University, Sapporo.

Primitive deutomerite widest a little below the septum or through the middle, tapering from the widest region and ending in a blunt extremity. Satellite deutomerite usually widest just below the septum.

Measurements of some associated individuals are as follows:

Table 1.

Length of Association (Primitive)	340	410	480	580
T L	170	210	245	310
L P	25	40	42	50
L D	147	173	210	262
WP	40	50	52	65
WD	50	80	85	120
LP:TL	1:6.8	1:5.3	1:5.8	1:6.2
WP:WD	1:1.3	1:1.8	1:1.6	1:1.8
(Satellite)				
t l	170	200	235	270
l p	20	25	25	40
l d	155	178	212	234
wp	45	50	80	80
wd	50	75	110	120
l p:t l	1:8.5	1:8.0	1:9.4	1:6.8
wp:wd	1:1.1	1:1.5	1:1.4	1:1.5

All dimensions are expressed in microns.

Endoplasm yellowish brown, dense. Nucleus spherical, 25—30 $\mu$  in diameter, with one nucleolus. Epimerite, a small spherical papilla situated upon the top of protomerite. Cysts and spores.

Cysts ellipsoidal, 210 $\mu$ x180 $\mu$  in average size. Spore ducts about 50 $\mu$  in length, less than the radius of cysts. Spores barrel shaped, 7 $\mu$ x4 $\mu$ , bluntly on both ends.

Remarks.

The organism from *Casida japana* Baly was found by M. Kimura in 1964 at a corner of the campus of Yamaguchi University. He observed its characters and noted it as *Gregarina* sp., however, this species may be identical with *Gregarina munieri*, because both have the common characteristics.

2. *Gregarina crenata* (Bhatia and Setna). (= *Caulocephalus japonicus* Hoshide).

Hosts: *Aulacophora femoralis* Motsch. Habitat: Intestine. Locality: Yamaguchi City, Sporadins. (Fig.2:a, b)

Biassociative, elongate cylindrical or ellipsoidal. Primitive protomerite longer than it is



Fig 1. *Gregarina munieri* (Schneider).

A mature association.

(Photo. by M. Kimura)

wide, widest just above, well rounded at the anterior end. Upper region of protomerite subglobular. Slight constriction at septum.

Primitive deutomerite elongate, cylindrical, widest at middle or a little above middle. Posterior end broad rounded or rather truncated.

Endoplasm dense, reddish brown. Nucleus spherical, 20—30 $\mu$  in diameter contains one nucleolus, divided into two different parts, vacuolated large part and compact small remainder.

Epimerite, a simple spherical knob or a tongue-shaped papilla.

Remarks.

The genus *Caulocephalus* was created by Bhatia and Setna in 1922 for the gregarine from *Aulacophora foveicollis* Kust in India. They emphasized that the epimerite was usually dilated anteriorly like a cauliflower. But Ray and Chatterjee (1936) inspected the same species captured at Calcutta and asserted that such a shape of the epimerite was produced artificially.

J. Théodoridès and P. Jolivet (1959) found, this parasite from beetles belonged to Chrysomelidae, Galerucidae, and Alticidae in Africa. This gregarine was transferred to the genus *Gregarina* and named *Gregarina crenata*.

The senior writer (H. Hoshide) (1951) found one gregarine from *Aulacophora femoralis* and considered it to be *Gregarina munieri*. Thereafter he (1957) distinguished it from the indian species mainly by the size of the body and named it *Caulocephalus japonicus*.

According to the kind suggestion of J. Théodoridès the writers examined the parasite from the same host this time and perceived that this species had almost the same characters that J. Théodoridès and P. Jolivet had presented.

The writers think that *Caulocephalus japonicus* belonged to the genus *Gregarina* and

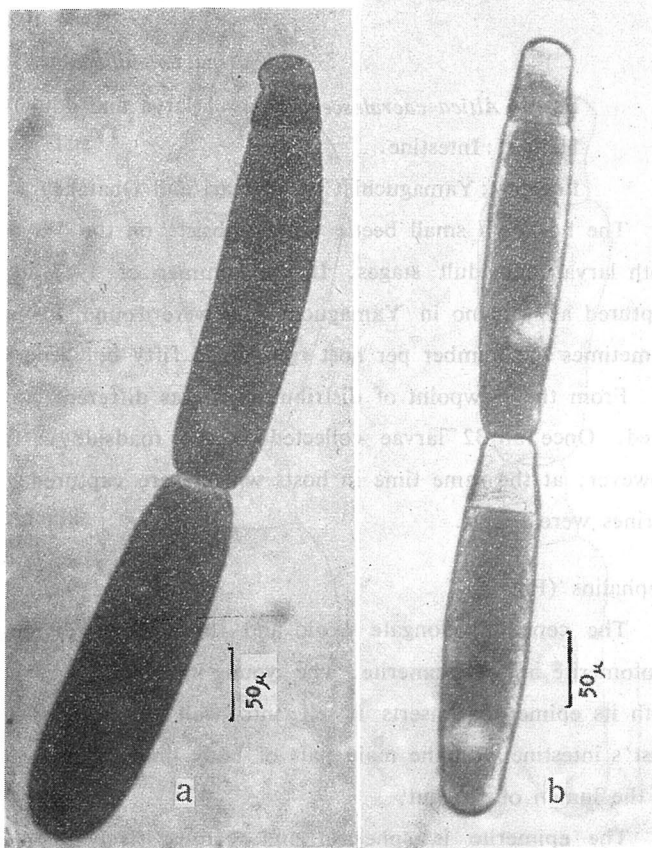


Fig. 2. *Gregarina crenata* (Bhatia and Setna).  
a, A mature association. b, Another slender one.

is identical with *Gregarina crenata* (Bhatia and Setna).

3. *Gregarina ampullaria* n. sp.

Hosts: *Altica caerulescence* Baly. (larva and adult)

Habitat: Intestine.

Locality: Yamaguchi City, Hikari and Obatake. (Yamaguchi prefecture).

The host is a small beetle which subsists on the leaves of *Acalypha australis* L. in both larval and adult stages. In the summer of 1967 about 60 per cent of the beetles captured at Miyano in Yamaguchi City were found to be parasitized with this species. Sometimes the number per host runs up to fifty but generally ten or more are found.

From the viewpoint of distribution it was different among the places where the host lived. Once all 32 larvae collected by the roadside at Yamaguchi City were infected, however, at the same time in hosts which were captured only 50 meters further, no gregarines were found.

Cephalins (Fig. 3)

The cephalins elongate ovoid and the body is divided into three parts; epimerite, protomerite and deutomerite. The young individual, with its epimerite, inserts itself into wall of the host's intestine with the main part of body liberated in the lumen of the gut.

The epimerite is spherical and comparatively large measuring about  $10\mu$  in diameter and is almost transparent. The outer membrane covering this part is very thin.

A cephalin is measured in microns as follows: TL 119, LP 47, LD 58, WP 30, WD 40, epimerite length 14, Nucleus diameter 15.

Sporadins (Fig. 4: a, b)

The grown cephalins detach from the gut wall and become free in its cavity and no longer have an epimerite. In the early stage of sporadins they are rather slender and cylindrical but as they grow older the body becomes wider.

The mature sporadins are biassociative, elongate ovoidal and like an ampulla in shape. The largest association observed was  $380\mu$  in length.

Primites: The protomerite is short and cylindrical with a shallow constriction through

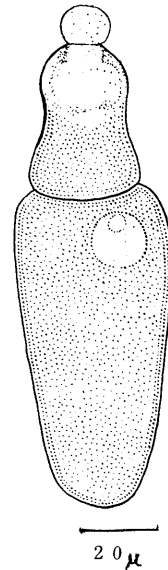


Fig. 3. *Gregarina ampullaria* n. sp.  
A cephalin with the epimerite at the top of the protomerite.

the anterior third. The anterior one third is globular and well rounded at the top. This part moves actively from side to side in the living animal. The posterior part from this constriction widens very gradually to the base where it is widest. The length of the protomerite is usually larger than the width and in some specimens the base of the protomerite projects into the anterior part of the deutomerite. The septum between the protomerite and deutomerite is distinct and there is a conspicuous constriction here.

The deutomerite is ovoid, widening rapidly from the septum, widest a short distance below the constriction at the septum and tapers gradually toward the posterior end, terminating in a well rounded extremity.

The epicyte is transparent and rather thick, measuring about  $2\mu$  in thickness at the deutomerite. There are differences in the endoplasm of deutomerite and protomerite. The endoplasm of protomerite is lighter and less dense than that of deutomerite. The anterior portion of protomerite is nearly transparent

devoid of granules and in the posterior portion just behind this transparent region some large clear granules are usually seen. The endoplasm of the posterior half of protomerite is almost as dense as that of the deutomerite. The granules in the deutomerite are very fine and dense. The body color is light brown.

The nucleus is spherical, measures about  $20\mu$  in diameter and contains one large nucleolus which is well stained with haematoxylin. The situation of the nucleus is variable, being located in the middle in some specimens. It is generally visible in vivo in young individuals but when the endoplasm is dense it difficult to see in living organism.

The interlocking device between primite and satellite is well developed the posterior end of primite fits intimately in the anterior portion of the satellite.

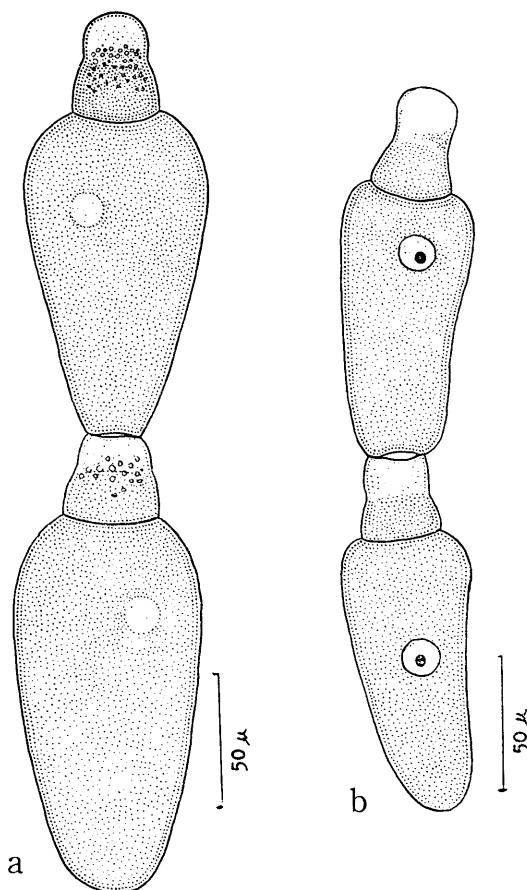


Fig. 4. *Gregarina ampulcaria* n. sp.  
a, A mature association. b, An association in the younger stage.

Measurements of *Gregarina ampullaria* n.sp. are shown as follows. All dimensions are expressed in microns.

Table 2.

Length of Association	235	250	290	350	315
(Primate)					
T L	105	120	140	140	170
L P	35	40	45	40	40
L D	72	85	95	104	132
W P	35	38	45	35	38
W D	50	65	70	55	65
LP : TL	1 : 3.0	1 : 3.0	1 : 3.1	1 : 3.5	1 : 3.8
WP : WD	1 : 1.4	1 : 1.7	1 : 1.8	1 : 1.7	1 : 1.7
(Satellite)					
t l	130	130	150	110	145
l p	28	35	35	30	30
l d	105	97	120	83	122
w p	28	35	40	28	40
w d	40	65	60	40	60
l p : t l	1 : 4.6	1 : 3.7	1 : 4.3	1 : 3.3	1 : 4.8
w p : w d	1 : 1.6	1 : 1.7	1 : 1.5	1 : 1.4	1 : 1.5

Syzygies from host larvae.

Table 3.

Length of Association	355	375	330	335	290
(Primate)					
T L	185	160	175	160	150
L P	50	50	55	45	35
L D	140	113	126	120	118
W P	45	50	50	35	40
W D	80	125	100	75	70
LP : TL	1 : 3.7	1 : 3.2	1 : 3.2	1 : 3.6	1 : 3.1
WP : WD	1 : 1.8	1 : 2.5	1 : 2.0	1 : 2.1	1 : 1.8
(Satellite)					
t l	170	215	155	175	150
l p	40	45	45	35	35
l d	134	75	120	142	118
w p	40	50	40	35	40
w d	75	100	50	70	60
lp : tl	1 : 4.3	1 : 4.8	1 : 3.4	1 : 5.0	1 : 4.3
w p : w d	1 : 1.9	1 : 2.0	1 : 1.3	1 : 2.0	1 : 1.5

Syzygies from host adults

Satellite: The satellite is nearly ovoid, and it is generally the same as the primite. But sometimes it is swelled and becomes spherical. The protomerite is somewhat shorter than the primite, when the width almost equals the height or is slightly wider than its length.

Cysts and Spores.

When the sporadins mature completely, each of the associated individuals gradually becomes spherical. The endocyte being filled with dense granules is nearly opaque. At the beginning of cyst formation the syzygy commences to revolve together. After rotating around a common axis for a while the two individuals come closer and closer together and finally form a spherical cyst.

The cysts are ovoidal, ellipsoidal or spherical, averaging  $126\mu \times 118\mu$  in size, with thin transparent outer envelope. The cysts are collected in the intestine or from the feces. Their dehiscence is by spore-ducts, measuring  $20\mu$  or so in length.

The spores are barrel-shaped and measure  $7\mu \times 4\mu$ .

Remarks.

This species may be clearly belonged to the genus *Gregarina* because of the shape of the epimerite, the character of the sporadins, cysts and spores.

Among the members of genus *Gregarina* it has a most intimate affinity with *Gregarina munieri* (Schneider) and *Gregarina crenata* (Bhatia aud Setna).

The shape of protomerite is very much like both *G. crenata* and this species. But the deutomerite of the latter is more obese than that of the former. With *G. munieri* this species somewhat resembles it in the shape and size of the deutomerite but in the shape of the protomerite and in the proportion: Length of protomerite to total length, both are much different.

This species is distinguished from both, the known *G. munieri* and *G. crenata* because it has an ampulla-shaped body in the sporadins stage and the ratio of protomerite length to total length is 1:3—4.

The authors want to propose that this species is a new member of the genus *Gregarina* and it will have intimate affinity with *G. munieri* and *G. crenata*.

#### SUMMARY

1. The gregarine from *Cassida japana*, which had been reported as *Grgarina* sp. by M. Kimura in 1964 is assigned to *Gregarina munieri* (Schneider).
2. *Caulocephalus japonicus* Hoshide 1957 is convinced of as a synonym of *Gregarina crenata* (Bhatia and Setna)
3. *Gregarina ampullaria* n. sp. is recorded from *Altica caerulescence* Baly in the intestine of both larvae and adults, in Yamaguchi prefecture, Japan.

The diagnosis of *G. ampullaria* n. sp. is as follows.

Sporadins, biassociative cylindrical to ovoidal and ampulla-shaped. Length of primate 100—170 $\mu$ , Width 50—70 $\mu$ , Ratio LP : TL=1 : 3—4, WP : WD=1 : 1.7. Protomerite short cylindrical, terminating well rounded extremity. Endoplasm dense, light brown. Nucleus spherical, 20 $\mu$  in diameter with one nucleolus. Cysts, various : spherical, ovoidal or ellipsoidal types : measuring 126 $\mu$  x 118 $\mu$ , dehiscence by short spore ducts. Spores barrel-shaped, measuring 7 $\mu$  x 4 $\mu$ .

#### REFERENCES

1. Bhatia, B. L., and Setna, S. B., (1924) On some new cephaline Gregarines. *Parasitology* 16: 179—288.
2. Foerster, H. : (1938) Gregarinen in schlesjschen Insekten. *Zeitsch. f. Parasitenk.*, 10: 157—209.
3. Hoshide, H. : (1951) Studies on the Gregarines from the Coleoptera in Japan. *Yamaguchi Jour. Sci.*, 2: 93—106.
4. Hoshide, H. : (1957) Studies on the cephaline Gregarines of Japan. II. 2) Description of the members belonging to the Family Gregarinidae. *Bull. Fac. Educ. Yamaguchi Uni.*, 7. Part II: 45—109.
5. Kimura, M. : (1964) On a Gregarine from *Cassida japana* Baly. (Unpublished results).
6. Labbé, A. : (1899) Sporozoa. *Das Tierreich.*, 5: 1—180.
7. Schneider, A. : (1876) Contribution a l'histoire des Gregarines des Invertebres de Paris et de Roscoff. *Arch. Zool. exp.*, 4: 493—604.
8. Thèodoridés, J. et Jolivet, P. : (1959) Eugregarines parasites de Coleopteres. *Explor. Parc. Nat. Albert*, 2 e. (8).
9. Watson, M. E. : (1916) Studies on Gregarines. *Ill. Biol. Monogr.*, II: 1—213.

#### EXPLANATION OF PLATE

1. A large association.
2. A large association, a different type of satellite is shown.
3. An association, early stage in developement.
4. A cephalin with epimerite.
5. Two sporadins in the process of rotation previous to cyst formation.
6. An ellipsoidal cyst.
7. A ripe cyst, a short spore-duct is seen at its upper side.
8. Several spores are being extruded in chains from the spore-duct.



