

Two New Species of *Unilobus* Théodoridès, Desportes and Jolivet, 1984 (Apicomplexa: Conoidasida) Parasitizing Tenebrionid Beetles along with the Remarks on the Genus and Its Family Status

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Summary. This paper deals with the morphology and life history of two new species of septate gregarines (Apicomplexa: Conoidasida: Gregarinidae) of the genus *Unilobus* Théodoridès, Desportes & Jolivet, 1984, obtained from two tenebrionid beetles of West Bengal, India. These are *Unilobus gonocephali* n. sp. from *Gonocephalum* sp. (Insecta: Coleoptera: Tenebrionidae) and *Unilobus scleroni* n. sp. from *Scleron irregulate* (Insecta: Coleoptera: Tenebrionidae). Total length of *U. gonocephali* varies from 200–467 µm. Gametocysts spherical, 410 µm in average diameter, dehisces through 7–9 sporoducts. *U. scleroni* is comparatively smaller, total length varies from 61–232 µm. Gametocyst spherical with average diameter 350 µm, dehices through 10 to 12 sporoducts. The two new species share traits which define the genus such as the absence of epimerite, expanded protomerite and late association. These two described species are not only different from the previously described species, but also oocysts (= spores) entirely different from each other in characters like measurements, number of sporoduct and in host range. This communication also confirmed the validity of the genus *Unilobus* Théodoridès, Desportes & Jolivet, 1984 and its placement under the family Gregarinidae Labbé, 1899.

Key words: *Unilobus*, septate gregarines, Apicomplexa, Conoidasida, tenebrionid beetles.

Abbreviations: TL – total length; LP – length of protomerite; LD – length of deutomerite; LN – length of nucleus; WP – width of protomerite; WD – width of deutomerite; WN – width of nucleus; PLUK – Protozoology Laboratory, University of Kalyani, West Bengal, India.

INTRODUCTION

Théodoridès and Desportes (1966) described a species of septate gregarines from *Cryphaeus gazelle* F.,

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a tenebrionid beetle from Laos, as *Anisolobus* sp. Later, this species was also found in the same host from North Vietnam by Théodoridès, Desportes and Jolivet (1984) and they established a new genus *Unilobus* to accommodate the species and renamed it as *Unilobus* (= *Anisolobus*) *cryphaei*. The genus *Unilobus* is characterized by sucker like and expanded protomerite, i.e. not multilobate whereas *Anisolobus* is characterized by a sucker like protomerite composed of unequal lobes, i.e. multi-

lobate. In addition to this, association is precocious in case of *Anisobolous*, whereas in case of *Unilobous* it is late. The absence of epimerite and the caudofrontal association are the characters shared by both genera *Unilobous* and *Anisobolous*. However, Levine (1984) was probably unaware of the latter paper and described the species as *Anisobolous theodoridesi*, which is in fact a synonym of *Unilobous cryphaei*. In the year 1987, Théodoridès and Jolivet described a second species of the genus *Unilobus* from an insect of East Africa (Cabo Verde Islands, Atlantic Ocean), *Alphitobius diaperinus*, as *Unilobus alphitobi*. However, in both cases cysts and spores were not observed. So the familial status of the genus *Unilobus* was uncertain.

It is interesting to note that, neither Levine (1988) in his classical monograph nor Clopton *et al.* (2002) in the "An illustrated guide to protozoa, 2nd ed.", the only modern comprehensive guide listing the septate gregarine genera and their synonyms, did not include the genus *Unilobus* Théodoridès, Desportes and Jolivet, 1984. They either did not consider *Unilobus* Théodoridès, Desportes and Jolivet, 1984 as a valid genus or unaware of the papers of Théodoridès *et al.* (1984, 1987).

In course of our studies on the cephaline gregarines from West Bengal, we have obtained two species of the genus *Unilobous* infecting two tenebrionid beetles, *Gonocephalum* sp. and *Scleron irregulate*. These are described here as new species as they differ markedly from each other and also from the previously described two species under the same genus.

MATERIALS AND METHODS

The adult insects *Gonocephalum* sp. and *Scleron irregulate* were collected approximately one a week from various grass fields of Halisahar (N23°, E88.5°) and Berhampore (N24°15', E88°26') respectively in the morning between 6 to 8 a.m. with the help of glass tube and brought alive to the laboratory for investigation from November, 1995 to February, 1998. A total of 206 adult *Gonocephalum* sp. 174 *scleron irregulate* were dissected and examined for the parasite. 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 (= spores) were studied by preparing oocyst (= spore) suspensions: a drop of freshly prepared Lugol's iodine solution (Lugol's iodine was prepared by adding 1 g each of KI and iodine crystals in 100 ml of distilled water) was placed on the oocyst (= spore) suspension

and the slide was examined under the oil immersion lens of a phase contrast microscope.

Figures of stained specimens were drawn with the aid of a mirror type 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 (= spores) were measured. Measurements were taken from widest part of protomerite, deutomerite, nucleus, gametocyst and oocyst (= spore) 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 of planes and solids used in this manuscript conforms to Clopton (2004).

RESULT AND DISCUSSION

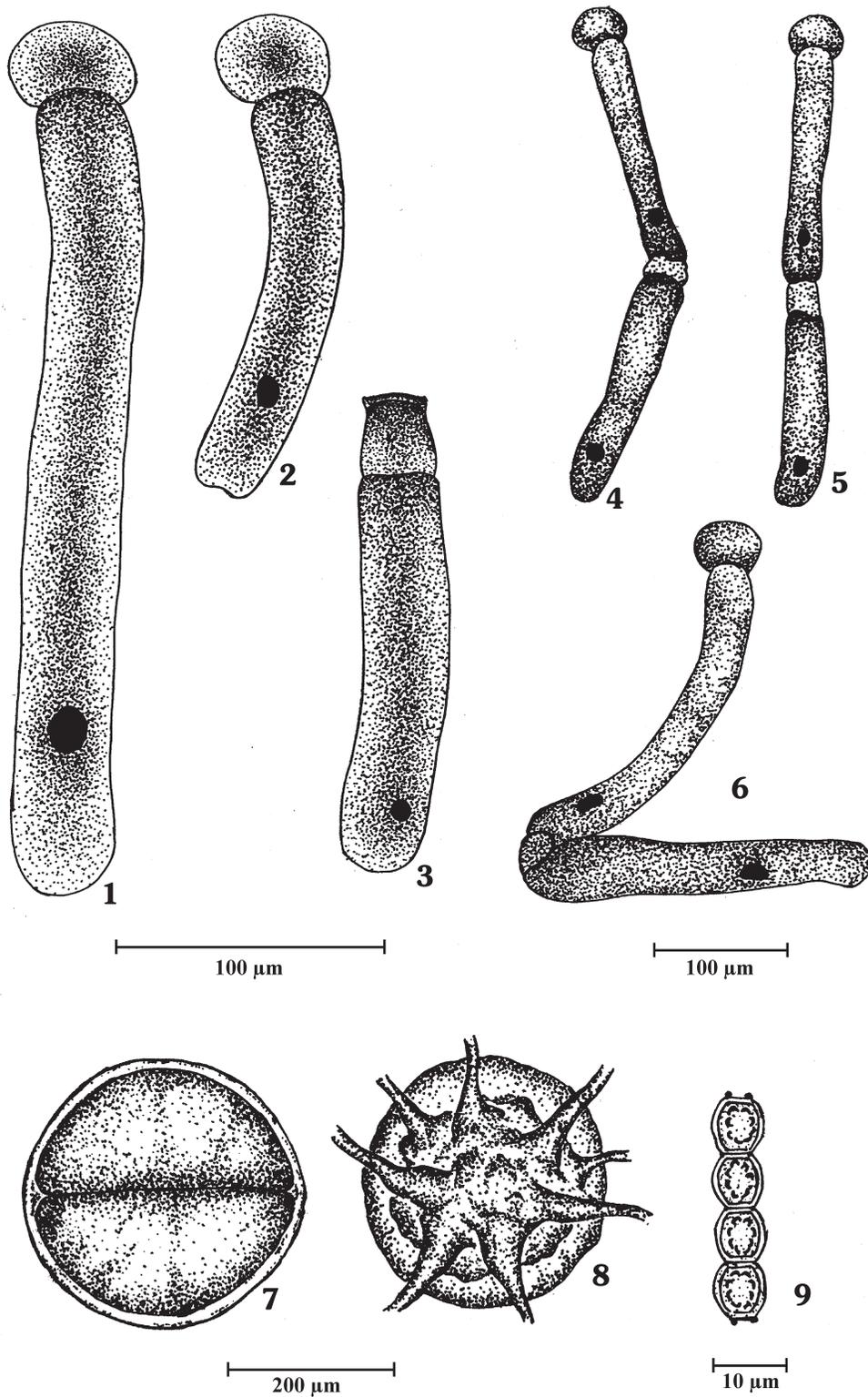
Unilobus gonocephali n. sp.

Trophozoite: Typical trophozoite stage is not found. Early stages are found to attach with the epithelial cells.

Gamont (Figs 1–3): Most of them are biassociative. A few solitary forms are also seen. The solitary gamonts are very narrowly oblong. Its total length varies from 192 to 467 µm. Protomerite is hemispherical or very broadly elliptoidal. In some forms it is somewhat oblong with a concavity at its tip (Fig. 3). A distinct septum separates protomerite from the deutomerite. It is also very narrowly oblong with more or less uniform width and rounded posterior extremity. The nucleus is mostly orbicular and situated generally at the posterior end of the deutomerite. Granulation of deutomerite is uniform but denser than protomerite.

Association (Figs 4–6): Late association, majority of the gamonts remain in biassociative and association is always caudo-frontal. The primite and the satellite are morphologically different. The protomerite of primite is hemispherical but the protomerite of satellite is oblong with a concavity or sucker like structure at its tip. This results in a firm attachment.

Gametocyst and oocyst (= spore) (Figs 7–9): Gametocysts are milky-white, more or less orbicular or spherical, isogamous (Fig. 7) and measure 365 to 490 µm in diameter (410 ± 22.1 , 30). The cyst dehisces by 7 to 9 long sporoducts after 72 hours of incubation inside the moist chamber (Fig. 8). The ducts are swollen at their bases, 40–60 µm in measure (51 ± 2.1 , 30) and its length varies from 140 to 175 µm (161 ± 7.2 , 30). Ectocyst is not observed. The oocysts (= spores)



Figs 1–9. Camera lucida drawings of different stages of *Unilobus gonocephali* n. sp. 1–3 – mature gamonts of different shapes and sizes; 4–6 – associations of different shapes and sizes; 7 – gametocyst; 8 – dehiscence of gametocyst with eight sporopores; 9 – dolioform oocysts (= spores) in chain.

are released in long chains (Fig. 9), shallowly or very shallowly dolioform. Each oocyst (= spore) is provided with a pair of small knob like structure at their two poles and measures $8.5\text{--}8.4 \times 7.3\text{--}7.1 \mu\text{m}$ ($8.3 \times 7.2 \pm$

$0 \times \pm 0, 50$). The sporozoites are sickle-shaped or very shallowly luniform and arranged in circular fashion within the oocyst (= spore).

Measurements (in micrometers)

The summary of measurements of fresh specimens of gamont, primite and satellite in association are given below:

Gamont:

TL = 200–467 ($338 \pm 17.1, 40$)

LP = 22–56 ($40 \pm 2.1, 40$)

LD = 178–422 ($277 \pm 14.1, 40$)

LN = 11–24 ($16 \pm 0.7, 40$)

WP = 36–73 ($53 \pm 2.8, 40$)

WD = 44–66 ($52 \pm 2.6, 40$)

WN = 9–17 ($13 \pm 0.6, 40$)

LP : TL = 1 : 6.24–14.4 ($8.6 \pm 0.2, 40$)

WP : WD = 1 : 0.71–1.56 ($1.05 \pm 0.06, 40$)

Primite in association:

TL = 198–465 ($336 \pm 16.9, 40$)

LP = 23–55 ($41 \pm 2.1, 40$)

LD = 175–421 ($278 \pm 14.2, 40$)

LN = 11–23 ($16 \pm 0.5, 40$)

WP = 40–71 ($53 \pm 2.4, 40$)

WD = 44–64 ($52 \pm 2.5, 40$)

WN = 10–18 ($13 \pm 0.4, 40$)

Satellite in association:

TL = 192–461 ($332 \pm 16.4, 40$)

LP = 21–54 ($39 \pm 2.2, 40$)

LD = 173–415 ($274 \pm 14.2, 40$)

LN = 10–23 ($16 \pm 0.6, 40$)

WP = 36–72 ($52 \pm 2.7, 40$)

WD = 43–64 ($51 \pm 2.6, 40$)

WN = 9–6 ($13 \pm 0.5, 40$)

Taxonomic summary:

Trophozoites: Typical trophozoite stage is not found.

Gamonts: Very narrowly oblong, mostly biassocia-tive, 338 μm in average total length.

Gametocysts: Milky-white, more or less orbicular, 410 μm in average diameter, dehisces by 7 to 9 long sporoducts.

Oocysts (= spores): Shallowly or very shallowly dolioform, $8.3 \times 7.2 \mu\text{m}$ in average dimensions.

Type material: Catalog No. H_{a4}–H_{a7} slides contain-ing syntypes have been deposited in the collection mu-seum of the PLUK.

Type locality: Halisahar (N23°, E88.5°) in the dis-trict of North 24-Parganas, West Bengal, India.

Type host: *Gonocephalum* sp. (Insecta: Coleoptera: Tenebrionidae).

Site of infection: Mid gut.

Symbiotype: Two whole specimens deposited at the Zoological Survey of India, Government of India, Kol-kata, India.

Prevalence of infection: 11.2% (23 out of 206) hosts are found to be infected. The infection is noticed during the winter period of year, i.e. from November to January. In other parts of a year the host insects popu-lation are either disappear from the field or very much reduced.

Etymology: The specific epithet *gonocephali* has been given after the generic name of the host insect.

***Unilobus scleroni* n. sp.**

Trophozoite (Figs 10–12): Typical tri-partite trophozoite stage is absent. Bi-partite intracellular stage with a protomerite and a deutomerite is also observed in sections.

Gamont (Figs 13–15): Early gamonts are found to attach with the epithelial cells (Fig. 11) but mature forms mainly remain in association. A few solitary forms are also encountered. Mature gamonts are cylindrical or very narrowly oblong measuring 61 to 232 μm in total length. Protomerite is hemispherical or very broadly elliptoidal, separated from deutomerite by a distinct septum. Deutomerite is cylindrical or very narrowly oblong with uniform granulation but denser than protomerite. Nucleus is spherical or orbicular, situated either in the middle or in the posterior end of the deutomerite.

Association (Fig. 16): Association is late and always caudofrontal. Both the primite and the satellite are morphologically similar except the shape of the

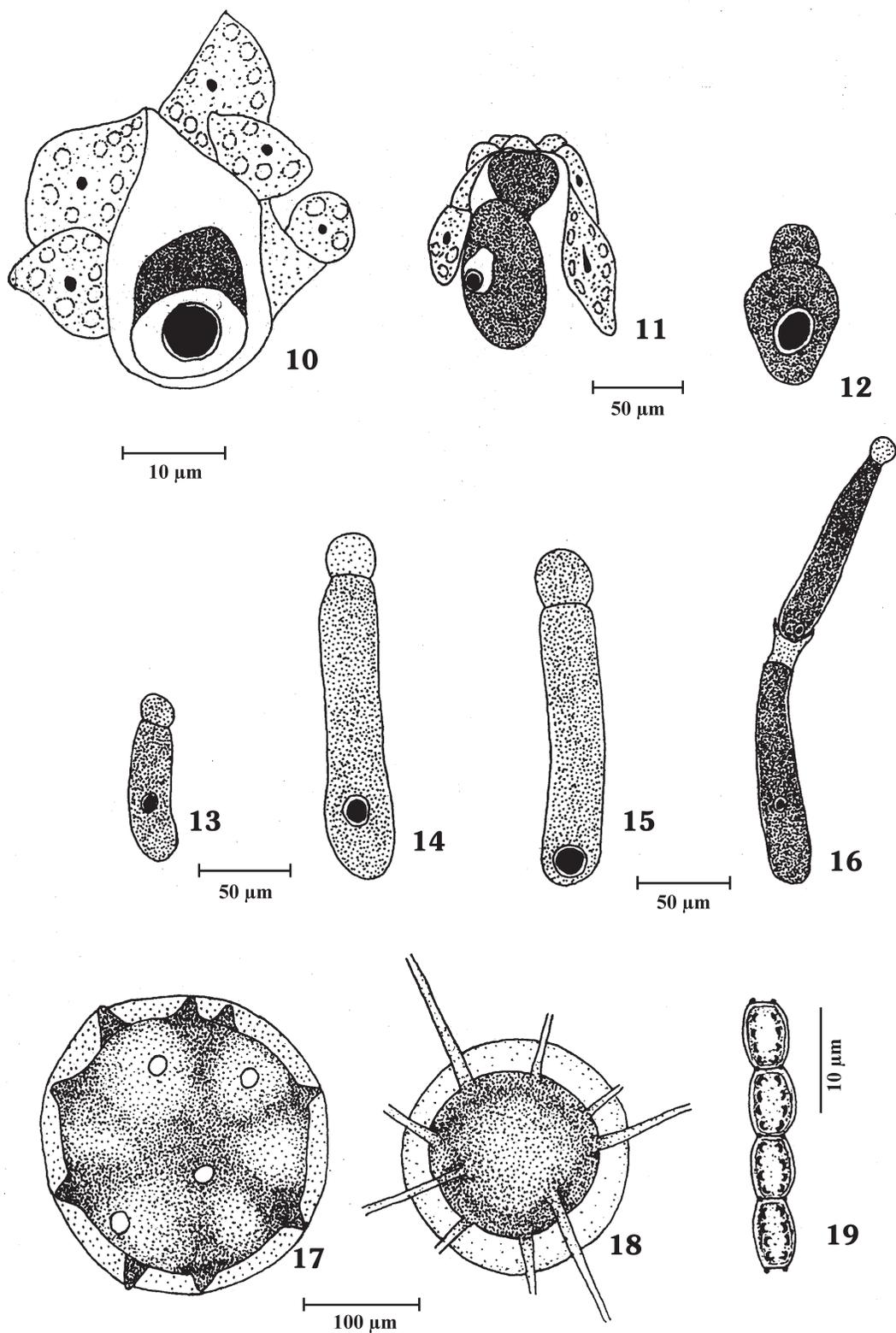
protomerite. The protomerite of primite is broadly elliptoidal but that of satellite is somewhat oblong with a concavity at its free end. This results in firm attachment between primite and satellite.

Gametocyst and oocyst (= spore) (Figs 17–19): Freshly collected gametocysts are orbicular, with a clear, thick, transparent and gelatinous ectocyst (Fig. 17). The ectocyst is irregular in outline with thickness varies from 37–42 μm (40 ± 0.4 , 30). Diameter of gametocyst varies from 338 to 360 μm (350.0 ± 1.1 , 30). The gametocyst dehisces by 10 to 12 long sporoducts after 107 h of incubation inside the moist chamber (Fig. 18). The length of the duct varies from 42 to 153 μm (120 ± 6.2 , 30). Dolioform oocysts (= spores) are released in long chains and are attached by a pair of papillate structure (Fig. 19), projecting from each pole. Each oocyst (= spore) measures $6.8\text{--}6.5 \times 4.8\text{--}4.6 \mu\text{m}$ ($6.7 \times 4.7 \pm 0.04 \times \pm 0.03$, 50). The sporozoites are sickle-shaped or very shallowly luniform and arranged linearly in two rows inside the oocyst (= spore).

Measurements (in micrometers):

The summary of measurements of fresh specimens of gamont, primite and satellite in association are given below:

Gamont:TL = 61–232 (212 ± 9.4 , 40)LP = 10–40 (26 ± 1.1 , 40)LD = 50–198 (141 ± 5.9 , 40)LN = 9–16 (12 ± 0.5 , 40)WP = 10–34 (23 ± 1.6 , 40)WD = 16–40 (29 ± 1.4 , 40)WN = 6–15 (11 ± 0.6 , 40)LP : TL = 1 : 5.0–8.3 (6.5 ± 0.3 , 40)WP : WD = 1 : 1.0–1.5 (1.3 ± 0.06 , 40)**Primite in association:**TL = 61–227 (212 ± 8.6 , 40)LP = 10–38 (26 ± 1.3 , 40)LD = 52–200 (142 ± 7.4 , 40)LN = 9–17 (12 ± 0.7 , 40)WP = 10–34 (24 ± 1.6 , 40)WD = 16–41 (30 ± 1.6 , 40)WN = 7–17 (12 ± 0.6 , 40)**Satellite in association:**TL = 63–233 (213 ± 8.1 , 40)LP = 12–41 (28 ± 1.2 , 40)LD = 55–208 (146 ± 7.5 , 40)LN = 9–16 (12 ± 0.6 , 40)WP = 10–35 (24 ± 1.7 , 40)WD = 17–42 (31 ± 1.5 , 40)WN = 7–17 ($11, \pm 0.5$, 40)



Figs 10–19. Camera lucida drawings of different stages of life cycle of *Unilobus scleroni* n. sp. **10** – early development of parasite in the epithelial tissue, showing that the development is intracellular; **11** – early gamont attached to the epithelial cells; **12** – gamont in section; **13–15** – gamonts of different ages, showing the position of the nucleus; **16** – gamont in caudo-frontal association; **17** – dehiscence of gametocyst; **18** – gametocyst with fully formed nine sporopores; **19** – oocysts (= spores) in chain.

Taxonomic summary:

Trophozoites: Typical tri-partite trophozoite stage is absent.

Gamonts: Very narrowly oblong, mostly biassocia-tive, 212 µm in average total length.

Gametocysts: Orbicular, 350 µm in average diam-eter, dehisces by 10 to 12 long sporoducts.

Oocysts (= spores): Dolioform, 6.7×4.7 µm in average dimensions.

Type material: Catalog No. H_{b1}–H_{b3}. 3 slides con-taining syntypes have been deposited at the collection museum of the PLUK.

Type locality: Berhampore (N24°15', E88°26') in the district of Murshidabad, West Bengal, India.

Type host: *Scleron irregulate* (Insecta: Coleoptera: Tenebrionidae).

Location in host: Mid gut.

Symbiotype: Two specimens deposited at the Zoo-logical Survey of India, Government of India, Kolkata, India.

Prevalence of infection: The hosts insects are available during the winter period of the year. On an average 71% (123 out of 179) of the hosts are found to be infected. Maximum infection recorded (81%) during the month of December–January.

DISCUSSION**A. The status of *Unilobous* as a valid genus**

Théodoridès, Desportes and Jolivet (1984) created a genus *Unilobous* to accommodate a species which was earlier described as *Anisolobous* sp. The type species is *Unilobous* (= *Anisolobous*) *cryphaei*. But they were unable to find any cyst and oocysts (= spores) of the gregarine. Perhaps due to these reasons neither Levine (1988) nor Clopton (2002) recognized this genus. However, in present study, complete life cycle of two species of the genus *Unilobous* has been worked out. Though the members of this genus share some common features with *Anisolobous*, like absence of epimerite and caudo-frontal association, but they possess a combination of unique features like: i) epimerite is absent; ii) expanded protomerite; iii) late caudo-frontal association; iv) deutomerite is cylindrical with almost constant width or with a minor variation: it gives the gamont a characteristic 'match-stick' like shape; v) gametocyst dehisces by multiple sporoducts; vi) oocysts

(= spores) are linearly arranged and ovoidal, dolioform or cylindrical in shape; and vii) restricted to tenebrionid beetles only. Considering all these characters collec-tively it can hardly be placed in any existing genera of septate gregarine. So the creation of the genus *Unilobus* by Théodoridès, Desportes & Jolivet is well justified.

B. Placement of the genus *Unilobus* under the family Gregarinidae Labbé, 1899

Presence of caudo-frontal association in the genus *Unilobous* tempted Théodoridès, Desportes and Jolivet (1984) to put the genus under the family Gregarini-dae. They did not find any cyst and spore. But without getting the cyst and oocysts (= spores), familial status of any gregarine cannot be confirmed. In addition to caudo-frontal association, the present investigation has confirmed that gametocysts are with sporoducts and oocysts are oval, elongate or cylindrical and symmetri-cal, and released in chains. These features undoubtedly place the genus *Unilobus* under the family Gregarini-dae Labbé, 1899.

C. Affinities

The characters like the absence of epimerite, ex-panded protomerite, late caudo-frontal association, sug-gest the inclusion of the described gregarines under the genus *Unilobus* Théodoridès, Desportes and Jolivet, 1984. Literature available reveals that there are only two species of *Unilobus*, *U. cryphaei* found simultane-ously from Africa (Zaire, Gabon) and Asia (Laos, Viet-nam) and *U. alphitobi* from East Africa (Cabo Verde Is-lands, Atlantic Ocean). However, in both cases cyst and spores are not found. But the present forms are entirely different from the earlier described species in general shape of the body, measurements, and in host range. However, the gamonts of the presently described two species are similar in shape; otherwise they are entirely different in other characters like measurements, num-ber of sporoduct and in host range. All these facts are sufficient to warrant them separate species status. These are designated as *Unilobus gonocephali* n. sp. and *U. scleroni* n. sp. respectively in this communication. The comparison of characters of the earlier two described species and the newly described two species are sum-marized in Table 1. It is interesting to note that these two species are frequently found in the host gut along with another septate gregarine of the genus *Stylocephalus*.

Table 1. Comparative characters of *Unilobus gonocephali* n. sp. and *U. scleroni* n. sp. with *U. cryphaei* and *U. alphitobi*.

| Characters | <i>Unilobus cryphaei</i> Théodoridès <i>et al.</i> 1984 | <i>U. alphitobi</i> Théodoridès and Jolivet, 1987 | <i>U. gonocephali</i> n. sp. | <i>U. scleroni</i> n. sp. |
|---------------------|--|---|---|---|
| 1. Epimerite | Absent | Absent | Absent | Absent |
| 2. Protomerite | Elongated and comparatively less expanded | Less elongated but massively expanded | Short but greatly expanded | Short but moderately expanded |
| 3. Gamont | Very large, mature form exceeds 500 µm, fibrillar sarcocytes are discernable | Comparatively small, narrowly oblong, measures 100 µm in average total length | Very large, narrowly oblong, measures 338 µm in average total length | Large, narrowly oblong, measures 212 µm in average total length |
| 4. Gametocyst | Not found | Not found | Milky white, orbicular, measure 365 to 490 µm in diameter. No ectocyst | Orbicular with a thick transparent ectocyst, diameter varies from 338 to 360 µm, transparent ectocyst present |
| 5. Sproduct | Not found | Not found | 7 to 9 sporoducts appear after about 72 h of incubation, each measures 161 µm in average length | 10 to 12 sporoducts appear after about 107 h of incubation, each measures 120 µm in average length |
| 6. Oocyst (= spore) | Not found | Not found | Dolioform, released in long chains, 8.3 × 7.2 µm in average | Dolioform, released in long chains, 6.7 × 4.7 µm in average |
| 7. Host | <i>Cryphaeus gazelles</i> | <i>Alphitobius diaperinus</i> | <i>Gonocephalum</i> sp. | <i>Scleron irregulate</i> |
| 8. Locality | Laos, Vietnam (Asia), Zaire, Gabon (Africa) | Cabo Verde Islands (Atlantic Ocean, East Africa) | India (Asia) | India (Asia) |

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