

Gregarina tibengae sp. n. (Apicomplexa: Eugregarinida) Described from *Zophobas atratus* Fabricius, 1775 (Coleoptera: Tenebrionidae)

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Summary. *Gregarina tibengae* sp. n. (Apicomplexa: Eugregarinida) is described from larvae and adults of *Zophobas atratus* (Coleoptera: Tenebrionidae). Trophozoites are solitary, attached to intestinal epithelium or located between epithelium and peritrophic membrane. Epimerites are spherical, ellipsoid, ovoid, or conical; protomerites are very broadly ovoid to depressed ovoid; deutomerites are obovoid to broadly obovoid. Association is precocious, caudofrontal and biassociative. Associations are located between intestinal epithelium and peritrophic membrane; early associations are axial-symmetric, late associations bilateral-symmetric. Primate protomerites are very broadly to depressed ovoid in early associations, broadly obovoid to very broadly obovoid in late associations. Primate deutomerites are obovoid to broadly obovoid, panduriform, with truncated posterior end. Satellite protomerites are very broadly ovoid to depressed ovoid; satellite deutomerites are obovoid to broadly obovoid, or cylindrical, panduriform, with truncated and emarginated posterior end. Total length of association 96 to 611 μm . Gametocysts are spherical, form 7-12 sporocysts. Oocysts are ellipsoid and measure 5.8 by 4.3 μm . They dehisce in chains.

Key words: Apicomplexa, cultured insects, Eugregarinida, *Gregarina tibengae* sp. n., Tenebrionidae, *Zophobas atratus*.

Abbreviations: AVEC-DIC - Allen video enhanced differential interference contrast, DIC - differential interference contrast, LD - deutomerite length, LE - epimerite length, LG - gametocyst length, LO - oocyst length, LP - protomerite length, ND - nucleus diameter, NL - nucleus length, NoD - nucleolus diameter, Pri - primate, Sat - satellite, TL - total length, TLA - total length of association, WD - deutomerite width, WE - epimerite width, WEO - equatorial oocyst width, WG - gametocyst width, WLO - width of oocyst ligaments, WP - protomerite width, ZSRO - Zoological Collection of the University of Rostock, Germany.

INTRODUCTION

Théodoridès and Jolivet (1990) described *Gregarina zophobasi*, a eugregarine parasite from the darkling beetle *Zophobas opacus* Sahlberg, 1823 in Brazil. In 1977 a related coleopteran, *Z. atratus* Fabricius, 1775 (syn. *Z. morio* Kraatz, 1880), was introduced from its habitat in Central and South America to Ger-

many by Pepe Alcaraz (Friederich and Volland 1992). It is now commercially cultured as a forage insect for insectivorous animals. The present study reports a new eugregarine species, *G. tibengae* sp. n., from the intestine of *Z. atratus* larvae and adults.

MATERIALS AND METHODS

Zophobas atratus larvae were purchased from commercial sources (Tropenwelt, Hamburg; Faunatopics, Marbach/Neckar; Germany) and subcultured by the author. Friederich and Volland (1992) give information on the cultivation of *Zophobas*. For this study the larvae

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were kept at 23–27°C in the dark. Rolled oats and pieces of apple and carrot were provided as food items.

Zophobas atratus larvae were eviscerated and intestines were dissected in Ringer's solution for insects (Seifert 1995) under a dissecting microscope. The gregarines were separated from the luminal content and transferred on cover slips using a pipette. They were fixed (2% glutaraldehyde in 0.05% Na-cacodylate buffer, pH 7.4) and hanging drop preparations were made. Measurements were taken using a micrometer; drawings were made with the aid of a drawing tube.

For permanent preparation wet smears were fixed in AFA (Seifert 1995) for 10 min, stained with Masson's haematoxyline according to Romais (1989) and counterstained in a 2% aqueous eosin solution, dehydrated in ascending ethanol concentrations, 100% n-propanol, Rotihistol (Carl Roth GmbH & Co, Karlsruhe, Germany) and mounted in neutral balsam. Gametocysts were collected from the intestine and the feces, transferred in Ringer's solution on cover slips and held in moist chambers for maturation and dehiscence. The obtained oocysts were fixed in 2% picric acid in Ringer's solution.

Photomicrographs of live gregarines in Ringer's solution were taken using a Zeiss Axiovert (bright-field, phase contrast) and a Reichert-Jung Polyvar (DIC). AVEC-DIC photomicrographs of oocysts were made using a Nikon Diavert with Hamamatsu Photonic Microscopy System C1966.

For morphometric measurements only gregarines from larvae of the last and last but one instar were used. Measurements were taken at their widest points, except for deutomerite lengths that were calculated as differences of total lengths and protomerite lengths. Measurements and indices in micrometers (μm) are given as range values followed by means, standard deviations, and sample sizes in parentheses. For the most part they are those used by Clopton (1998). Terminology of shapes follows that suggested by the Systematics Association Committee for Descriptive Biological Terminology (1962).

RESULTS

Taxonomic position: Eugregarinida Léger, 1900 *sensu* Levine *et al.* (1980); Septatina Lankester, 1885 *sensu* Levine *et al.* (1980); Gregarinicae Chakaravarty, 1960; Gregarinidae Labbé, 1889 *sensu* Levine (1988), with characters of the genus *Gregarina* Dufour, 1828 *sensu* Clopton (2002): epimerite conical, button-shaped, globular, or cylindrical; association precocious; oocysts doliform, navicular, or spherical; in intestine of insects.

Gregarina tibengae sp. n.

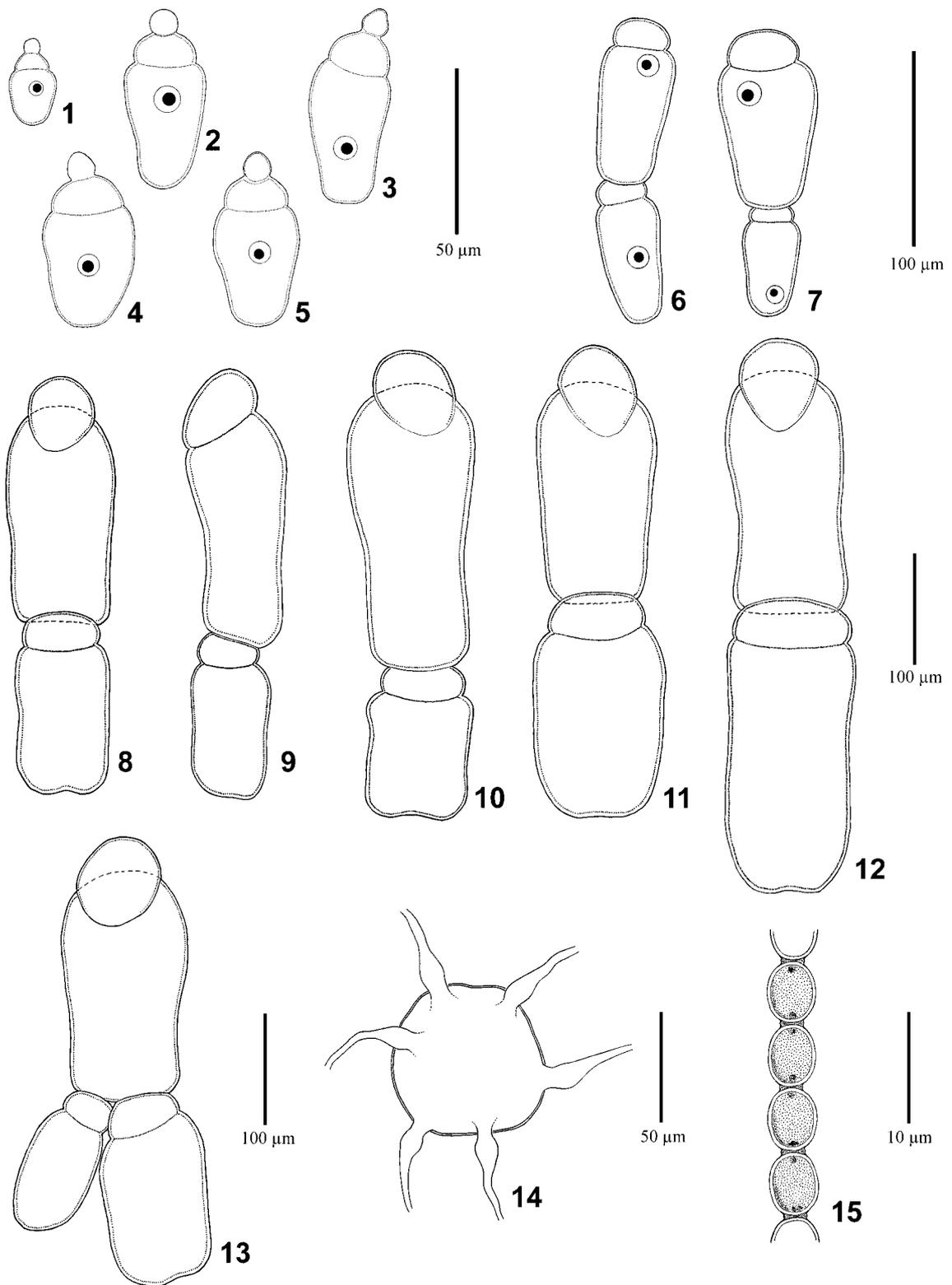
Trophozoite (Figs 1-5, 17): Cephalont (Figs 1-5; measurements and indices given in Table 1) attached to host intestinal epithelium by epimerite, in posterior half of mesenteron. Solitary, never in association. Earliest cephalonts circular to broadly ovoid (Fig. 1), minor constriction at protomerite-deutomerite septum. Older cephalonts more oblong. Epimerite simple, spherical

(Fig. 2), ellipsoid to broadly ovoid (Fig. 5), or cone-shaped (Figs 3, 4); without diamerite; surface smooth, without folds or impressions. Protomerite very broadly to depressed ovoid, tapered anteriorly to junction with epimerite; moderate posterior constriction at protomerite-deutomerite septum. Deutomerite shape obovoid to broadly obovoid, with shallow sinuate margin at half to second third of length on each side, rounded to truncated posteriorly, maximum width at anterior third; septum concave. Nucleus spherical; typically with single spherical nucleolus, eccentric in location, young cephalonts occasionally with 2-3 nucleoli. Sporont (Fig. 17) solitary; free, located between host intestinal epithelium and peritrophic membrane; morphology similar to late cephalonts but epimerite absent; epimerite residue absent.

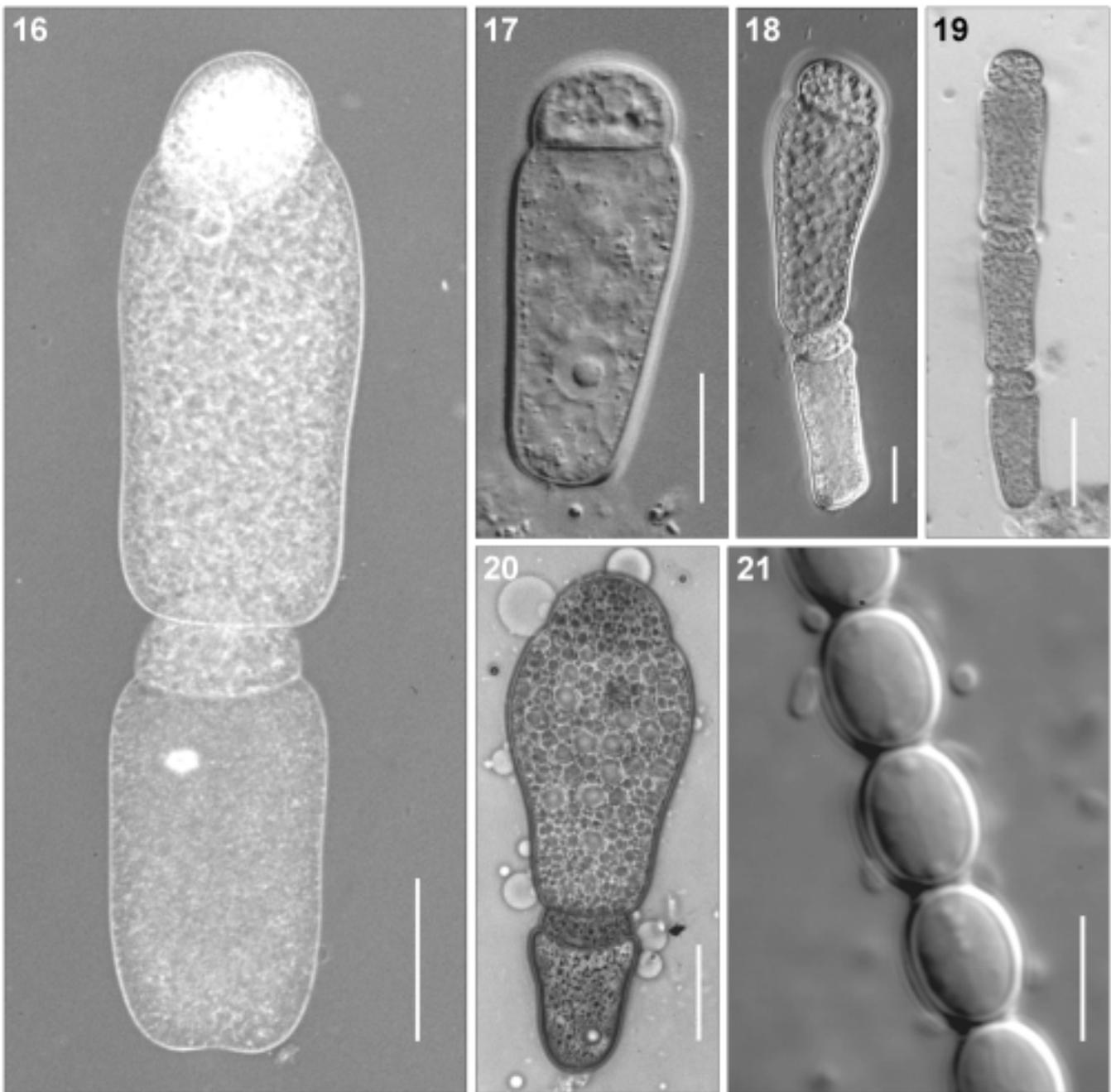
Association (Figs 6-13, 16, 18-20): Measurements and indices given in Table 1). Syzygy early (precocious), caudofrontal; multiple associations of one primate with two satellites occasionally observed (Fig. 13), linear multiple association seen only once (Fig. 19); located between host intestinal epithelium and peritrophic membrane, in second third of mesenteron of host. Early associations axial-symmetric (Figs 6, 7), morphology of gamonts similar to that of sporonts, some associations with noticeable small satellites (Fig. 20). Late associations bilaterally symmetric, dorsoventrally flattened, the side bearing the contact areas between primate protomerite and primate deutomerite as well as between primate deutomerite and satellite protomerite is (hereby) defined as "ventral" (on the left in Fig. 9).

Primate: Protomerite very broadly to depressed ovoid in early associations; or broadly to very broadly obovoid in late associations, situated on ventral side; with moderate constriction at protomerite-deutomerite septum. Deutomerite obovoid to broadly obovoid; anterior end rounded; septum concave; with shallow to moderate sinuate margin at half to second third of length on each side (panduriform); maximum width typically in anterior third, in some late associations anterior and posterior third with similar width, maximum width rarely in posterior third, close to the posterior end (never more than 10 μm wider than anterior third); truncated posteriorly. Nucleus spherical; abaxial in location; in all parts of deutomerite, mostly in anterior half; concealed by granula in late associations; with one spherical nucleolus, eccentrically located.

Satellite: Protomerite very broadly to depressed ovoid; contact area with primate deutomerite ventral; rounded anteriorly; with minor constriction at protomer-



Figs 1-15. *Gregarina tibengae* sp. n. **1-5** - cephalonts; **6-7** - early associations; **8, 10-12** - late associations, ventral view; **9** - late association, lateral view; **13** - multiple association; **14** - dehiscent gametocyst with sporoducts; **15** - oocysts.



Figs 16-21. *Gregarina tibengae* sp. n. **16** - late association (bright-field, inverted); **17** - sporont (DIC); **18** - early association (DIC); **19** - linear multiple association (DIC, inverted); **20** - early association (phase-contrast, inverted); **21** - oocysts (AVEC-DIC). Scale bars 5 μ m (21); 20 μ m (17, 18); 50 μ m (19, 20); 100 μ m (16).

ite-deutomerite septum. Deutomerite of early associations obconoid to obovoid; with shallow sinuate margin in middle to second third of length; rounded or truncated posteriorly. Deutomerite of late associations obovoid to

broadly obovoid or cylindrical, often with square outline (Fig. 10); septum concave; with shallow to moderate sinuate margin in middle of length; truncated and emarginated posterior end, sinus elongated anteriorly

Table 1. Measurements (in μm) and indices of trophozoites and associations of *G. tibengae* sp. n.

Measurements	Trophozoites Cephalonts	Primites	Associations	Satellites
LE	2-11 (6 \pm 1.7; 78)			
WE	5-10 (7 \pm 1.2; 78)			
WE/LE	0.60-3.00 (1.27 \pm 0.41; 78)			
LP	3-22 (12 \pm 4.1; 78)	15-99 (54 \pm 23.8; 202)		6-61 (30 \pm 13.0; 202)
WP	11-35 (20 \pm 5.2; 78)	25-91 (57 \pm 18.4; 202)		13-135 (62 \pm 29.5; 202)
WP/LP	1.31-3.67 (1.83 \pm 0.44; 78)	0.77-1.93 (1.15 \pm 0.29; 202)		1.31-3.29 (2.07 \pm 0.33; 202)
LD	12-89 (39 \pm 15.1; 78)	51-263 (141 \pm 52.0; 202)		24-258 (125 \pm 58.0; 202)
WD	13-55 (27 \pm 7.9; 78)	31-138 (82 \pm 29.9; 202)		15-157 (74 \pm 35.2; 202)
WD/LD	0.45-1.33 (0.71 \pm 0.14; 78)	0.41-0.79 (0.59 \pm 0.08; 202)		0.39-0.91 (0.59 \pm 0.09; 202)
TL	20-119 (57 \pm 20.1; 78)	66-343 (195 \pm 74.4; 202)		30-309 (154 \pm 70.4; 202)
LE/TL	0.06-0.21 (0.11 \pm 0.03; 78)			
LP/TL	0.14-0.29 (0.21 \pm 0.03; 78)	0.18-0.38 (0.27 \pm 0.04; 202)		0.12-0.30 (0.19 \pm 0.03; 202)
LD/TL	0.57-0.78 (0.69 \pm 0.04; 78)	0.63-0.82 (0.73 \pm 0.04; 202)		0.70-0.95 (0.81 \pm 0.03; 202)
LP/LD	0.20-0.50 (0.30 \pm 0.05; 78)	0.22-0.60 (0.37 \pm 0.08; 202)		0.14-0.42 (0.24 \pm 0.04; 202)
WP/WD	0.64-0.95 (0.77 \pm 0.06; 78)	0.59-0.91 (0.71 \pm 0.07; 202)		0.65-1.00 (0.84 \pm 0.07; 202)
ND	4-11 (6 \pm 1.6; 54)	8-32 (17 \pm 6.1; 102)		4-28 (13 \pm 5.0; 81)
NoD	2-4 (3 \pm 0.8; 52)	3-12 (7 \pm 2.2; 102)		2-11 (6 \pm 2.2; 81)
TLA			96-611 (345 \pm 137.5; 202)	
Pri TL/TLA			0.46-0.77 (0.57 \pm 0.05; 202)	
Sat TL/TLA			0.30-0.55 (0.44 \pm 0.05; 202)	
Pri WD/Sat WD			0.75-2.57 (1.20 \pm 0.31; 202)	
Pri WD/Sat WP			0.90-2.95 (1.44 \pm 0.34; 202)	

boldface type - mean

into shallow longitudinal groove. Nucleus spherical; abaxial in location, in all parts of deutomerite, mostly in posterior half; concealed by granula in late associations; with one spherical nucleolus; eccentrically located.

In developing associations satellites smaller than primites (Figs 8-10); in mature associations satellites reach or exceed primate length and width. Index Pri TL/TLA decreases in large associations; satellite size increases in

Table 2. Characters of late associations of *G. zophobasi* and *G. tibengae* sp. n. Measurements in μm .

Character	<i>G. zophobasi</i>	<i>G. tibengae</i> sp. n.
Primate		
Maximum TL	> 400	343
WD	50	31-138 (82)
Protomerite	anterior dilatation, ampullaceous or “champignon” aspect, situated on anterior pole	very broadly obovoid, without anterior dilatation, situated on ventral side
Satellite		
Maximum TL	> 300	309
WD	70	15-157 (74)
Protomerite	quadrangular, fine transversal epicytical striation	depressed ovoid, striation absent
Deutomerite	attenuated posterior end	emarginated posterior end
Protomerite-deutomerite septum	triangular	concave
Nucleus	ellipsoid, > 30 μm in length, always in posterior part of deutomerite	spherical, up to 32 μm in diameter, in all parts of deutomerite, mostly in anterior part

boldface type - mean; >: exceeding

Table 3. Measurements (in μm) and characters of species similar to *G. tibengae* sp. n. 1 - *G. amoji*, 2 - *G. ceropriae*, 3 - *G. decourti*, 4 - *G. dragescoi*, 5 - *G. gonocephali*, 6 - *G. inclinata*, 7 - *G. jolivetii*, 8 - *G. maculata* var. *banulensis*, 9 - *G. mesomorphi*, 10 - *G. niphandrodes*, 11 - *G. steini*, 12 - *G. tibengae*

Character	1	2	3	4	5	6	7	8	9	10	11	12
Associations												
TLA	-	-	-	> 1000	> 175	355-470	-	-	-	-	-	96-611 (345)
Pri TL	-	?-200	[> 300]	430-550	78-91	160-203	165-300	115-165	250-300	143-377 (233)	108-187 (147)	66-343 (195)
Pri WD	-	45	-	-	36-38	63-85	> 300	-	108-149	102-235 (146)	13-33 (20)	31-138 (82)
Pri L/P/TL	-	-	-	-	0.17-0.22	0.17-0.30	-	-	0.17	0.17-0.40 (0.26)	0.10-0.18 (0.13)	0.18-0.38 (0.27)
Sat TL	-	?-300	[> 350]	445-540	78-91	181-260	150-400	115-145	250-300	102-347 (218)	84-141 (113)	30-309 (154)
Sat WD	-	45	-	-	33-40	52-85	-	-	108-149	61-214 (114)	11-22 (15)	15-157 (74)
Sat L/P/TL	-	-	-	-	0.12-0.18	0.11-0.17	-	-	0.13	0.15-0.33 (0.19)	0.10-0.17 (0.13)	0.12-0.30 (0.19)
Bilateral symmetry	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Curved	no	yes	yes	no	yes	yes	no	yes	yes	yes	no	yes
Margine of deutomerites	sinuate	sinuate	convex	sinuate	sinuate or convex	sinuate	convex	sinuate	convex	convex	sinuate	sinuate
Panduriform deutomerites in late associations	yes	no	no	no	no	no	no	yes	no	no	yes	yes
Emarginated posterior end of satellite deutomerite	no	no	no	no	no	no	no	no	no	no	no	yes
Nuclei	spherical	ellipsoid	spherical	ellipsoid	spherical	spherical	ellipsoid	ellipsoid	spherical	spherical	spherical	spherical
Pri NL; Sat NL	-	30; 30	-	-	8-16; 8-16	20; 20	40-50; 40-50	-	34; 30	36; 36	18; 18	32; 28
Gametocysts												
Shape	ellipsoid	ellipsoid	-	-	-	spherical or ellipsoid	spherical	-	spherical	spherical	ellipsoid	spherical
Diameter	70 x 54	140-200	-	-	-	200	150	-	200	22.5	85 x 60 - 160 x 100 ²	146-191 (167)
Oocysts:												
LO x WO	4.4 x 2.3	4 x 2	-	-	-	6 x 4	6 x 3	-	5.6 x 4.2	8.8 x 5.5	4.9 x 4.9 ¹ , 5 x 3 ²	5.8 x 4.3

Boldface - means; > - exceeding; [] - not given by the authors, assumed from illustration; ¹ from Clopton *et al.* (1991); ² from Geus (1969)

large associations; indices Pri WD/Sat WD and Pri WD/Sat WP constantly decreasing during growth of association.

Cytology: Cytoplasm brownish; primitive cytoplasm always filled with numerous large vesicles; vesicles absent in satellite cytoplasm (Fig. 18); paraglycogene granula present in both cells.

Gametocysts (Fig. 14): Roughly spherical; with hyaline gametocyst coat, coat thickness (in Ringer's solution) 2-6 μm ; located between host intestinal epithelium and peritrophic membrane, egested with feces. Gametocysts isolated from intestine: LG (including coat) 139-176 (161 ± 9.1 , 21), WG 129-173 (153 ± 11.1 , 21), WG/LG 0.87-1.0 (0.95 ± 0.04 , 21). Gametocysts collected from feces: LG 146-191 (167 ± 15.0 , 15), WG 173-187 (159 ± 15.2 , 15), WG/LG 0.90-1.0 (0.96 ± 0.03 , 15). Gametocysts collected from fresh feces mature and dehisce within 50-80 h. Oocysts extruded in chains through sporoducts. Sporoducts with wide basis, proximal bulbous and distal tube; length 25-50 μm ; usually 7-12 per gametocyst, sometimes more primordial but not evaginated.

Oocysts (Figs 15, 21): Ellipsoid, connected in chains by "ligaments"; uniform in size and shape; LO 5.7-6.2 (5.8 ± 0.13 , 64), WEO 4.0-4.5 (4.3 ± 0.11 , 64), WLO 1.7-2.3 (2.1 ± 0.1 , 64), WEO/LO 0.68-0.77 (0.74 ± 0.02 , 64), WLO/LO 0.27-0.39 (0.36 ± 0.02 , 64), WLO/WEO 0.38-0.54 (0.48 ± 0.03 , 64); cross-section roughly circular; two, sometimes numerous, polar oocyst residua present.

Taxonomic summary

Type specimens: Eight syntype slides (19 March 2002-17 October 2002) were deposited in the ZSRO (# ZSRO Pro 0001a - Pro 0001h; label: *Gregarina tibengae* (Apic., Eugregarinida) ex. *Zophobas atratus* Fabricius, 1775 (Col., Tenebrionidae), [syntype], leg. & det. Martin Jahnke, Germany: Rostock, cultured host).

Type host: *Zophobas atratus* Fabricius, 1775 (Tenebrionidae: Tenebrioninae: Tenebrionini); larvae and adults

Symbiotype: One adult host specimen was deposited in the ZSRO (# ZSRO Pro 0001i; label: Germany, Rostock, 2003-V-12, leg. & det. Martin Jahnke, Tenebrionidae, *Zophobas atratus* Fabricius, 1775, cultured, [symbiotype], type host of *Gregarina tibengae*). Two additional voucher specimens were labeled analogous and have got collection numbers ZSRO Pro 0001j - ZSRO Pro 0001k.

Infection site: Mesenteron.

Prevalence: High; 83% (n=30).

Type locality: Germany, Rostock

Other localities: Germany: Hamburg, Marbach (Neckar), Tübingen; cultured.

Etymology: The specific epithet refers to the Swabian word "Tibenga" for Tübingen where the author found this gregarine for the first time.

DISCUSSION

The present gregarine is the second eugregarine species described from *Zophobas*. A comparison of the characters of *Gregarina zophobasi* Théodoridès et Jolivet, 1990 and *G. tibengae* is given in Table 2. In both species the gamonts of late associations are alike in size. However, *G. zophobasi* can be distinguished from *G. tibengae* based on its elongated gamonts, the "champignon" view of primitive protomerites, the attenuated posterior end of deutomerite, the triangular shape of the protomerite-deutomerite septum, and the ellipsoid nucleus which is always situated in the posterior part of deutomerite. Of the 56 species of *Gregarina* known from Tenebrionid beetle hosts, the following 11 species exhibit a similar primitive protomerite: *G. amoji* Datta, Ghose et Haldar, 1991, *G. ceropriae* Théodoridès et Desportes, 1967, *G. decourti* Théodoridès, Desportes et Jolivet, 1975, *G. dragescoi* Théodoridès et Desportes, 1966, *G. gonocephali*, Obata, 1953, *G. inclinata* Hoshide, 1979, *G. joliveti* Théodoridès, 1958, *G. maculata* Léger, 1904 var. *banyulensis* Théodoridès, 1955, *G. mesomorphi* Devdhar et Deshpande, 1971, *G. niphandrodes* Clopton, Percival et Janovy, 1991, and *G. steini* Berndt, 1902. A comparison of these species with *G. tibengae* is given in Table 3. The associations of all species are bilateral-symmetric and most are slightly curved, similar to those of *G. tibengae*. *Gregarina decourti*, *G. joliveti*, *G. mesomorphi*, and *G. niphandrodes* can be distinguished from *G. tibengae* based on their convex margins of deutomerites. In *G. gonocephali* only early associations have deutomerites with sinuate margins whereas those of late associations are convex. *Gregarina amoji*, *G. ceropriae*, *G. dragescoi*, *G. inclinata*, *G. maculata* var. *banyulensis*, and *G. steini* exhibit sinuate margins of deutomerites but only *G. amoji*, *G. maculata* var. *banyulensis*, and *G. steini* possess panduriform deutomerites similar to those of *G. tibengae*. *Gregarina amoji* lack the emarginated posterior end

of the satellites, moreover, *G. amoji* has ellipsoid gametocysts. *Gregarina ceropriae*, *G. maculata* var. *banyulensis*, and *G. steini* can be distinguished from *G. tibengae* based on their attenuate deutomerites. *Gregarina inclinata* possess characteristic unciform associations different from *G. tibengae*. In comparison with *G. tibengae* the associations of *G. dragescoi* are plainly larger (maximum TLA 611 µm versus >1000 µm). The combination of diagnostic characters of the described gregarine, especially associations possessing bilateral symmetry, panduriform margin of deutomerites, and emarginated posterior end of satellite deutomerite, seem to differentiate it from known *Gregarina* species of Tenebrionidae.

Acknowledgements. I thank Raimund Apfelbach (University of Tübingen), Willfried Kröger (University of Rostock) and Willi Maile (Freising) for their kind support in microscopy and Susanne Braun (Tübingen) and Axel Sonnemann (Hohenstein) for obtaining host larvae for this study.

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Received on 12th June, 2003; revised version on 14 September, 2004; accepted on 15th September, 2004