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Gregarina niphandrodes N. Sp. (Apicomplexa: Eugregarinorida) from Adult *Tenebrio molitor* (L.) with Oocyst Descriptions of Other Gregarine Parasites of the Yellow Mealworm

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ABSTRACT. *Gregarina niphandrodes*, a new species of septate eugregarine, is described from the adult mealworm, *Tenebrio molitor*. Measurements given are means in μm. Gamonts in association. Nuclear diameter consistent, 35.5. Primite: protomerite hemispherical; length 59.5; width 91.2; deutomerite ovoid; length 173.4; width 145.6; total length 232.9. Length of protomerite/total length index 25.7. Length of deutomerite/total length index 74.3. Length of protomerite/length of deutomerite index 35.4. Width of protomerite/width of deutomerite index 63.5. Satellite: protomerite hemispherical; length 40.2; width 81.6; deutomerite ovoid; length 177.4; width 113.9; total length 217.6. Length of protomerite/total length index 19.0. Length of deutomerite/total length index 81.0. Length of protomerite/length of deutomerite index 23.8. Width of protomerite/width of deutomerite index 76.7. Gametocysts spherical, diameter 225, producing multiple oocyst chains under dry storage in 36–52 h. Oocysts very uniform in shape and size, dorsum doliform with angles squared by enclosing sheath, length 8.8, width 5.5; flattened dorsoventrad with mesad tumidus, pleuron height 2.2. Morphological measurements, indices, and oocyst descriptions are given for *G. cuneata*, *G. polymorpha* and *G. steini*.

Key words. Eugregarinorida, *Gregarina*, parasite, *Tenebrio*.

FOUR eugregarine species, *Gregarina cuneata* [31], *G. steini* [1], *G. polymorpha* [9, 31], and *Steinina ovalis* [31], have been described from the larvae of *Tenebrio molitor* (L.), the yellow mealworm [1, 9, 31]. *Gregarina cuneata* has been reported from both larval and adult mealworms; however, no species have been described strictly from adult *T. molitor* [13, 22, 34]. The economy and availability of the host as a research animal has made this parasite complex a model for research on gregarine pathogenesis [10, 32, 33], ultrastructure and development [4, 5, 24, 25], and ecology [23, 27, 28]. The gregarine parasites of *T. molitor* provide an excellent opportunity for comparative study markedly improved by the addition of a species that is evidently restricted to adult host forms. This study describes *Gregarina niphandrodes* n. sp. from adult *T. molitor*, and compares the morphology of fixed and fresh specimens.

MATERIALS AND METHODS

Samples of adult *Tenebrio molitor* were taken from colonies of indefinite origin maintained at the School of Biological Sciences, University of Nebraska–Lincoln. *Gregarina niphandrodes* n. sp. was encountered regularly during periodic survey dissections during the year before data was collected for this description. Five adult beetles were dissected in insect saline without sucrose [2] and examined for parasites in May 1990. Twenty free trophozoites were measured from each fresh preparation. Additional specimens were fixed in alcohol-formalin-acetic acid (AFA) for permanent slide preparations. Fixed specimens were washed in 70% ethanol, stained with Semichon's aceto-carmine, dehydrated in ethanol, cleared with xylene, and mounted in Canada balsam. Measurements were taken from 100 fresh and 100 balsam-mounted trophozoites. Twenty additional beetles were dissected in insect saline without sucrose [2] and examined for parasites in March 1991. Thirty-six free gamont associations were measured. In each of these dissections, numerous trophozoites were present, free in the lumen, and attached to the epithelium. Widths of protomerites and deutomerites were taken at the widest points. The same parasite was found in hundreds of adult beetles, taken from these and other colonies at the University of Nebraska, dissected over a period of 11 months (May 1990–March 1991).

Larvae from the same colonies were dissected to provide representative examples of *Gregarina cuneata*, *G. polymorpha*, and *G. steini*. Fresh measurements (n = 29, 22, and 20, respectively) were taken from free gamont associations of each species. Measurements and ratios from *G. cuneata*, *G. polymorpha*, and *G. steini* were compared with those of *G. niphandrodes* using Fisher's Student's *t*-test for equality of means, corrected for unequal variance when indicated by an *F*-test for equality of variance [30].

Measurements presented in this paper are range values with embedded means followed by the standard deviation in parentheses. All measurements are in micrometers unless otherwise indicated. Data and observations are from live specimens unless otherwise indicated.

Gametocysts were collected by isolating 50 adult *T. molitor* in a clean plastic shoe box with a moist paper towel for 24 h. Individual gametocysts were placed in deep-well slides for maturation. Cysts held in dry storage sporulated and produced initial oocyst chains within 23–36 h. Oocyst chain extrusion appeared complete within 36–52 h. Oocyst morphology and dimensions were taken from fresh preparations of extruded oocyst chains. Oocyst preparations were made in water and in glycerin. Glycerin preparations allowed free rotation of the oocyst, facilitating the elucidation of oocyst morphology. Individual oocyst lengths and widths were measured at their widest points. Oocysts displayed remarkable similarity in shape and size.

Drawings were made from living and preserved protozoa with the aid of a camera lucida. All observations were made on an American-Optical Spencer binocular compound microscope with 15× wide field eyepieces, 40× objective, and a Silge & Kuhne Ortho-Illuminator B. Green filters were used for measuring and daylight filters for observing color in living and preserved specimens.

RESULTS

Description

Gregarina niphandrodes n. sp. (Fig. 1–4)

Diagnosis. Gregarinidae with the characters of the genus *Gregarina* as defined by Dufour [7] and revised by Watson [34], Kamm [13], and Levine [19]: bioassociative sporonts, simple globular, conical, button-shaped or cylindrical papilla-like epimerite, dehiscence of cysts by sporoducts, and doliform, navicular, or spherical oocysts.

The following abbreviations and indices are used in this paper: LP, length of protomerite; LD, length of deutomerite; TL, total length; WP, width of protomerite; WD, width of deutomerite; PLI, (LP/TL); DLI, (DL/TL); P/DLI, (LP/LD); P/DWI, (WP/WD). Each ratio was multiplied by 100 to yield an index value.

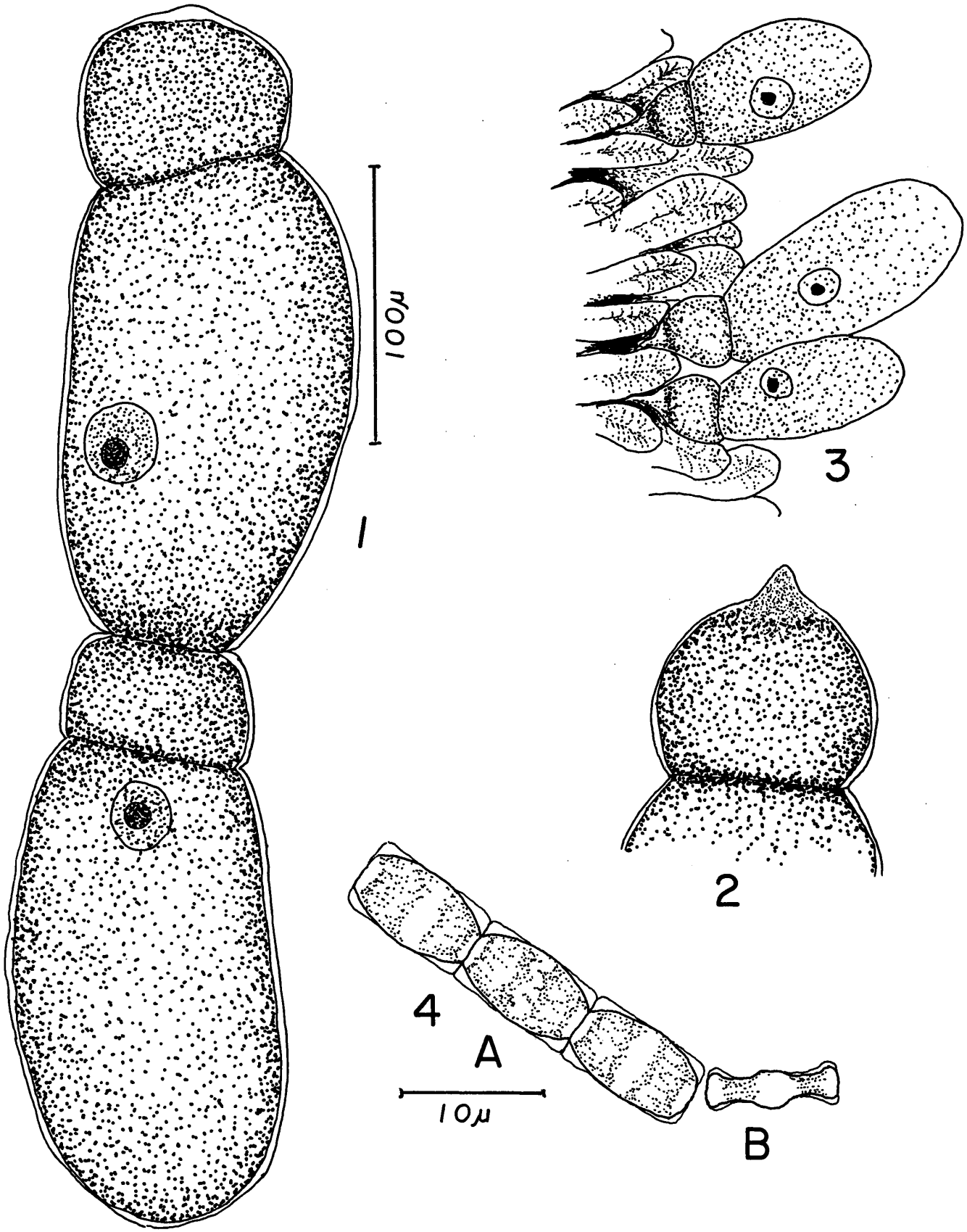

Fig. 1–4. *Gregarina niphandrodes* n. sp. 1. Gamonts in association. 2. Protomerite and epimerite showing general form and lack of apparent septum. 3. Detail of ventricular epithelium with attached trophozoites. 4. Oocyst chain showing details of dorsad (A) and mesad (B) aspects.

Table 1. Mean values, ranges, and standard deviations (SD) of the morphological measurements and ratios of associated gamonts of *Gregarina cuneata* with *t*-test results for comparisons with homologous measurements and ratios of *G. niphandrodes* n. sp. (Data taken from life; mean values presented as ranges with embedded means; all measurements in micrometers.)

	Mean	SD	t_{obs}	Degrees of freedom
	Primites			
LP	38.40–**63.39**–115.20	17.47	0.88	63.00
WP	24.00–**76.13**–76.80	10.84	15.58[a]	63.00
LD	86.40–**189.02**–278.40	55.81	1.21	63.00
WD	38.40–**15.05**–105.60	15.05	14.64[a]	56.00
TL	124.80–**252.41**–364.80	69.27	1.22	63.00
PLI	17.39–**25.61**–32.00	0.04	0.10	63.00
DLI	68.00–**74.39**–82.61	0.04	0.10	63.00
P/DLI	21.05–**34.80**–47.06	0.07	0.24	62.00
P/DWI	40.00–**69.09**–100.00	0.12	2.16[a]	45.00
	Satellites			
LP	19.20–**40.88**–86.40	15.48	0.20	42.00
WP	28.80–**46.68**–67.20	10.41	10.68[a]	61.00
LD	38.40–**132.74**–211.20	42.55	3.77[a]	63.00
WD	43.20–**76.14**–105.60	15.96	5.37[a]	49.00
TL	67.20–**173.63**–297.60	54.62	3.17[a]	63.00
PLI	15.00–**23.95**–42.86	0.05	4.18[a]	63.00
DLI	57.14–**76.05**–85.00	0.05	4.19[a]	63.00
P/DLI	17.64–**32.23**–74.99	0.11	3.59[a]	47.00
P/DWI	50.00–**61.60**–74.99	0.08	3.68[a]	44.00

[a] Significantly different from *G. niphandrodes*; $P > |t| = 0.05$.

Trophozoite (Fig. 1, 2). Protomerite hemispherical; length 18.0–**63.9**–132.6 (19.6+); width (mesad) 51.0–**87.6**–132.6 (20.1+). Deutomerite ovoid; length 91.8–**185.4**–336.6 (58.8+); width (mesad) 61.2–**115.9**–214.2 (35.2+); total length 132.6–**249.3**–428.4 (73.2+). Indices: PLI 17.4–**26.0**–43.3 (4.7+); DLI 56.7–**74.0**–82.6 (4.7+); P/DLI 21.1–**35.7**–76.5 (9.2+); P/DWI 33.3–**78.0**–100.0 (11.6+). Epimerite globular to subconical (Fig. 1); without visible septum; obvious in young trophozoites, apparent in some form in all trophozoite stages. Mesenteron epithelium often crowded with attached parasites in a range of sizes (Fig. 2). Fresh trophozoites with endocyte clear to partially granular when very young, becoming fully opaque with maturity. Ectocyte clear to light in color. Color whitish under dissecting microscope with incandescent light, clear to orange brown under compound microscope with daylight filter. Nucleus diameter variable with maturity, 19.0–28.8; location variable, usually in anterior half of deutomerite.

Gamonts in association (Fig. 3). Bioassociative, although associations relatively rare, even among large individuals. Association often precocious; occurring in extracellular growth of trophozoite. Fresh gamont associations with endocyte uniformly granular and opaque with ectocyte clear to delineate a pellicular border 3.0 wide, pellicle 2.2 thick; color whitish under dissecting microscope with incandescent light, orange brown to black under compound microscope with daylight filter.

Primite. Protomerite hemispherical; length 40.8–**59.5**–102.0 (18.0+); width (mesad) 71.4–**91.2**–122.4 (12.7+). Deutomerite ovoid; length 91.8–**173.4**–295.8 (48.4+); width (mesad) 102.0–**145.6**–234.6 (27.9+); total length 142.8–**232.9**–377.4 (60.0+). Indices: PLI 16.7–**25.7**–40.0 (5.3+); DLI 60.0–**74.3**–83.3 (5.3+); P/DLI 20.0–**35.4**–66.7 (10.7+); P/DWI 50.0–**63.5**–75.0 (7.7+). Epimerite absorbed into protomerite, apical complex faintly apparent. Nuclear diameter consistent, 35.5; usually off-center in anterior third of deutomerite.

Satellite. Protomerite hemispherical; length 20.4–**40.2**–61.2 (8.8+); width (mesad) 6.12–**81.6**–122.4 (15.8+). Deutomerite ovoid; length 81.6–**177.4**–295.8 (51.0+); width (mesad) 61.2–**113.9**–214.2 (38.3+); total length 102.0–**217.6**–346.8 (56.2+). Indices: PLI 14.7–**19.0**–33.3 (4.1+); DLI 66.7–**81.0**–85.3 (4.1+); P/DLI 17.2–**23.8**–50.0 (7.2+); P/DWI 50.0–**76.7**–150.0 (23.2+). Epimerite satellite lost in association. Nuclear diameter consistent, 35.5; usually slightly off-center in posterior third of deutomerite.

Gametocysts. In life (and fixative) white and spherical, diameter approximately 205; hyaline coat thickness ~10, increasing diameter to approximately 225. Gametocysts shed and stored under dry conditions sporulate in 24-36 h, forming 1–3 oocyst chains in 36-52 h.

Oocysts (Fig. 4A, B, 15). Dorsum doliform with angles squared by enclosing sheath, length 8.8, width 5.5; flattened dorsoventrad with mesad and terminal tumidi, pleuron height 2.2; uniform in size and shape.

Type host. *Tenebrio molitor* (L.); yellow mealworm.

Type locality. Mealworm colonies, School of Biological Sciences, University of Nebraska–Lincoln, Lincoln, Lancaster County, Nebraska, USA.

Specimens deposited. Mixed trophozoites, mature gamonts, and gamont associations. Hapantotypes (three slides, USNM No. 81568) have been deposited in the United States National Museum Helminthological Collection, Biosystematic Parasitology Laboratory, Beltsville, Maryland.

Infection site. In adults only, in the mesenteron between cardiac and pyloric valves, infection centered in anterior ventriculus (beneath dorsal fat bodies), between epithelium and peritrophic membrane.

Etymology. The specific epithet, *niphandrodes*, is derived from the Greek *niphas* and given to mark the similarity in form between single trophozoites and the snowmen of the senior author's Nebraska childhood.

Remarks. *Gregarina niphandrodes* is found only in adult *T. molitor* whereas *G. polymorpha, G. steini,* and *Steinina ovalis* are reported only from larvae; *G. cuneata* is reported from both larval and adult stages [29, 34]. *Gregarina niphandrodes* is easily separated from the established gregarine fauna of *T. molitor* using morphological measurements and ratios, oocyst morphology and general appearance.

Steinina ovalis can be differentiated from *G. niphandrodes* using family characters. *Steinina ovalis* is an actinocephalid with characters of the family as defined by Levine [20]: frontal association producing gametocysts without sporoducts, dehiscing by simple rupture; oocysts biconical, cylindrodiconical, or irregular. In contrast *G. niphandrodes* displays fronto-caudal association producing gametocysts with one to three sporoducts, dehiscing in oocyst chains; oocysts doliform.

Gregarina niphandrodes is distinguished from other *Gregarina* infecting *T. molitor* by general morphological characters that are real and stable in the literature over time and space. The protomerite is hemispherical in *G. niphandrodes,* spatulate in *G. cuneata,* ovoid in *G. polymorpha,* and ovoid and offset in *G. steini.* The deutomerite is elliptical and widest through the center in *G. niphandrodes,* elongate and widest through the posterior in *G. cuneata,* elongate and cylindrical in *G. polymorpha,* and pear-shaped and widest at the septum in *G. steini.*

The primites of *G. niphandrodes* are comparable in length with those of *G. cuneata,* but are significantly wider (Table 1).

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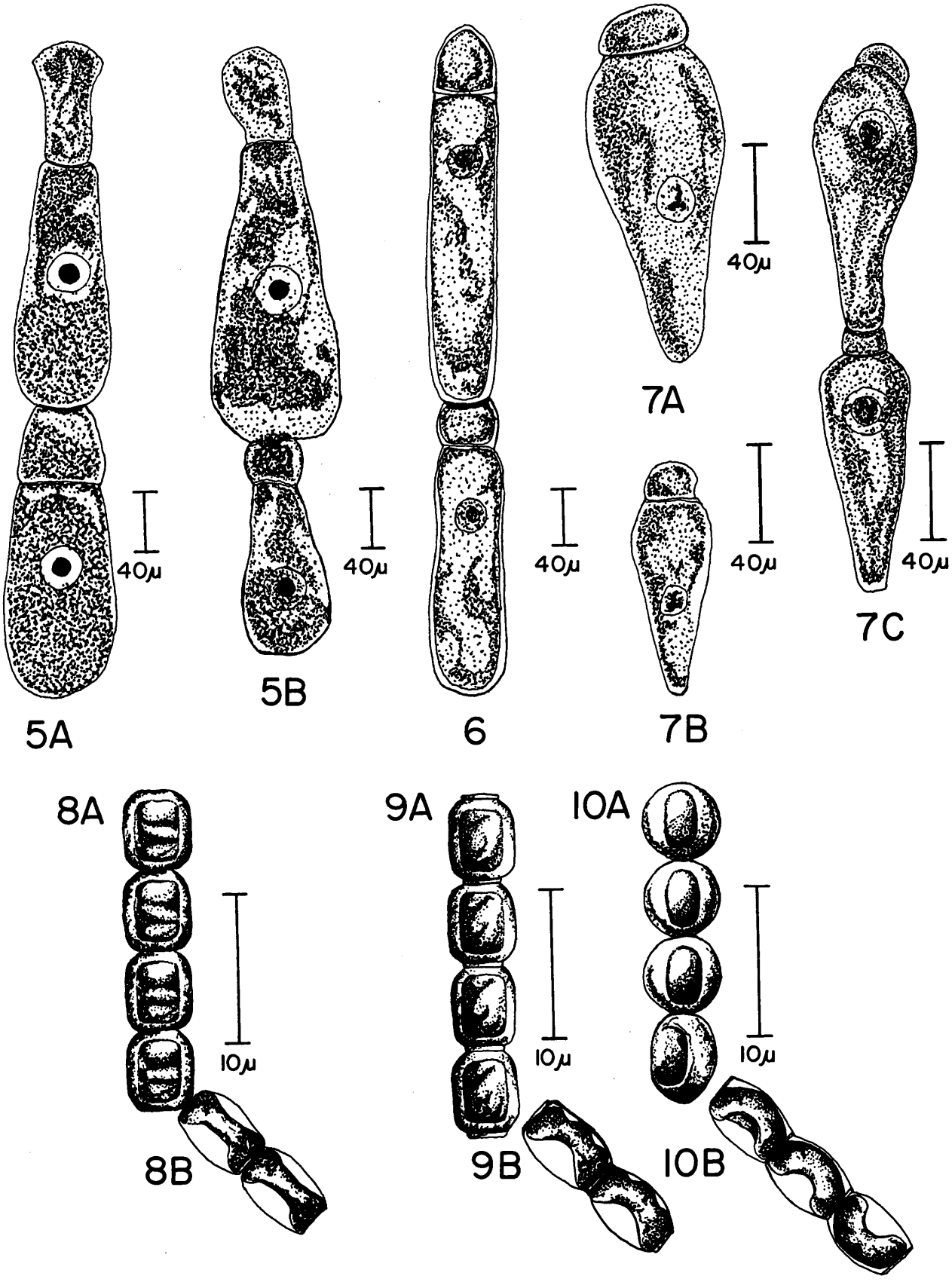
Fig. 5–10. *Gregarina* of *Tenebrio molitor.* 5–7. Gamonts in association. 5. *G. cuneata,* (A) redrawn from Watson [26], (B) redrawn from Berndt [1], details added from life. 6. *G. polymorpha,* redrawn from Watson [26], details added from life. 7. *G. steini,* (A, B) redrawn from Berndt [1], details added from life, (C) drawn from life. 8–10. Oocyst chains showing details of dorsad (A) and mesad (B) aspects. All oocyst chains drawn from life. 8. *G. cuneata.* 9. *G. polymorpha.* 10. *G. steini.*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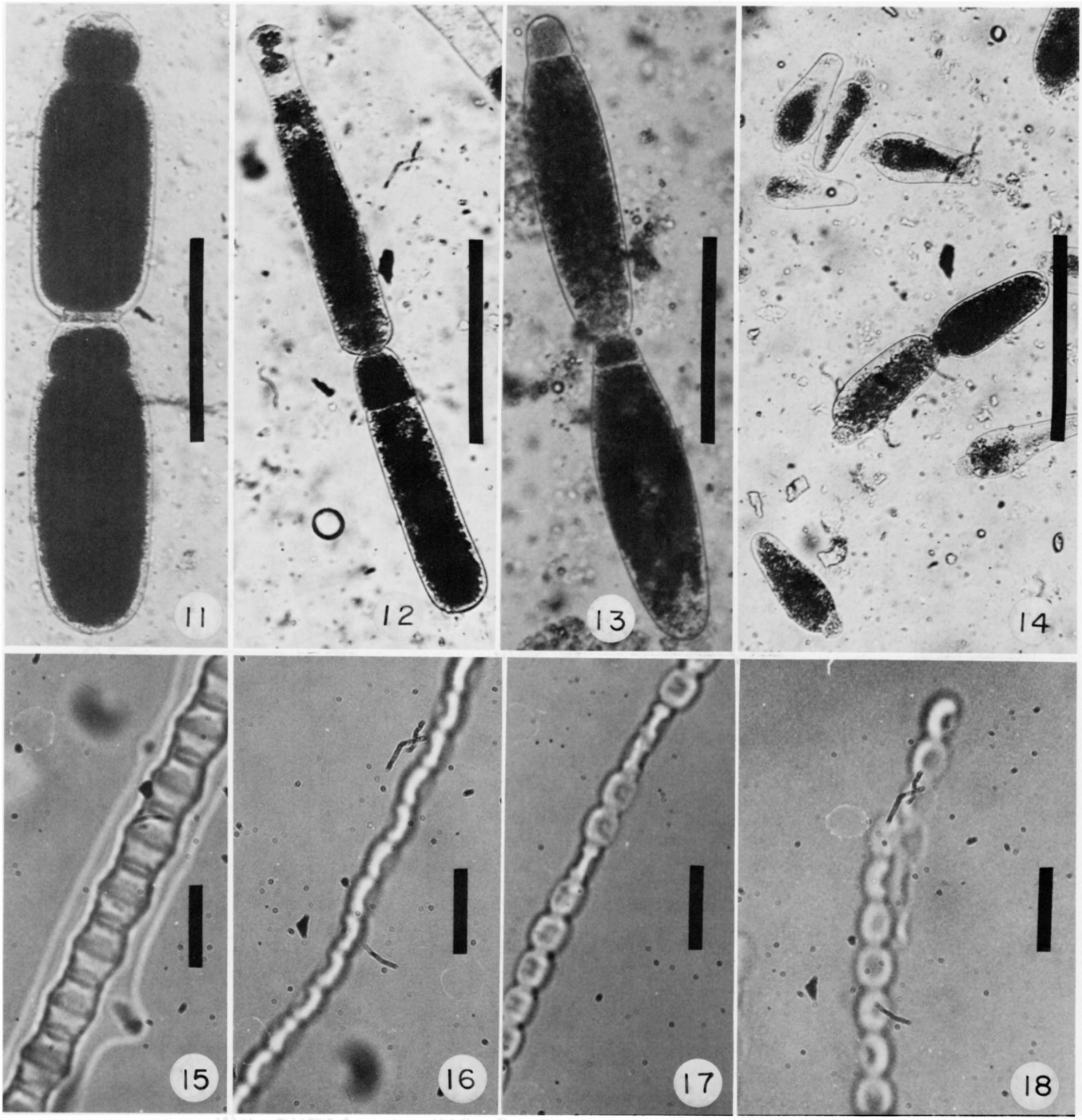

Fig. 11–18. *Gregarina* gamont associations and oocysts of species infecting *Tenebrio molitor*. **11–14.** Gamonts in association (scale bar = 160 micrometers). **11.** *G. niphandrodes* n. sp. **12.** *G. cuneata*. **13.** *G. polymorpha*. **14.** *G. steini*. **15–18.** Oocysts (scale bar = 10 micrometers). **15.** *G. niphandrodes* n. sp. **16.** *G. cuneata*. **17.** *G. polymorpha*. **18.** *G. steini*.

The satellites of *G. niphandrodes* are significantly different from those of *G. cuneata* in all morphological measurements and ratios except protomerite length (Table 1). The nuclear diameter of *G. cuneata* is smaller than *G. niphandrodes*, 24 versus 35.5. Nuclear placement in the associated gamonts of *G. cuneata* is uniform, mesad in the anterior third of the deutomerite: nuclear placement in associated gamonts of *G. niphandrodes* is variable, usually off-center in the anterior third of the deutomerite in primites, usually off-center in the posterior third of the deutomerite in satellites. *Gregarina niphandrodes* is further differentiated from *G. cuneata* by pellicular thickness and the appearance of the ectocyte. *Gregarina niphandrodes* has an ectocyte 3.0 thick. There is no apparent ectocyte in fully mature specimens of *G. cuneata*. The pellicle of *G. niphandrodes* is thicker than

Table 2. Mean values, ranges, and standard deviations of the morphological measurements and ratios of associated gamonts of *Gregarina polymorpha* with *t*-test results for comparisons with homologous measurements and ratios of *G. niphandrodes* n. sp. (Data taken from life; mean values presented as ranges with embedded means; all measurements in micrometers.)

	Mean	SD	t_{obs}	Degrees of freedom
	Primites			
LP	24.20–**37.50**–55.00	7.07	5.46[a]	56.00
WP	24.20–**36.80**–52.80	7.09	18.44[a]	56.00
LD	143.00–**219.90**–363.00	59.43	3.10[a]	38.00
WD	46.20–**66.55**–101.20	15.82	12.10[a]	56.00
TL	167.20–**257.40**–418.00	65.84	1.45	56.00
PLI	11.63–**14.82**–18.60	0.02	11.47[a]	44.00
DLI	81.40–**85.18**–88.37	0.02	11.47[a]	44.00
P/DLI	13.16–**17.44**–22.86	0.02	9.71[a]	40.00
P/DWI	44.44–**56.07**–68.18	0.06	3.79[a]	56.00
	Satellites			
LP	19.80–**28.60**–55.00	7.08	5.25[a]	56.00
WP	24.20–**39.50**–61.60	9.71	12.57[a]	56.00
LD	130.40–**212.40**–341.00	60.72	2.36[a]	56.00
WD	50.60–**70.00**–110.00	17.36	5.95[a]	53.00
TL	123.20–**241.00**–387.20	66.32	1.44	56.00
PLI	9.38–**12.18**–16.18	0.02	8.47[a]	54.00
DLI	83.82–**87.82**–90.62	0.02	8.47[a]	54.00
P/DLI	10.34–**13.92**–19.30	0.03	7.49[a]	48.00
P/DWI	39.29–**57.09**–70.83	0.09	4.49[a]	51.00

[a] Significantly different from *G. niphandrodes*; $P > |t| = 0.05$.

Table 3. Mean values, ranges, and standard deviations of the morphological measurements and ratios of associated gamonts of *Gregarina steini* with *t*-test results for comparisons with homologous measurements and ratios of *G. niphandrodes* n. sp. (Data taken from life; mean values presented as ranges with embedded means; all measurements in micrometers.)

	Mean	SD	t_{obs}	Degrees of freedom
	Primites			
LP	13.20–**19.91**–33.00	4.48	12.52[a]	42.00
WP	22.00–**29.37**–39.60	3.99	27.00[a]	46.00
LD	94.60–**126.72**–154.00	15.97	5.29[a]	47.00
WD	37.40–**49.94**–63.80	7.39	19.35[a]	43.00
TL	107.80–**146.63**–187.00	19.55	7.90[a]	47.00
PLI	10.34–**13.49**–17.65	0.02	12.62[a]	46.00
DLI	82.35–**86.51**–89.66	0.02	12.62[a]	46.00
P/DLI	11.54–**15.64**–21.43	0.02	10.60[a]	41.00
P/DWI	50.00–**59.32**–72.00	0.07	2.02	54.00
	Satellites			
LP	11.0–**15.07**–22.00	3.29	15.37[a]	49.00
WP	15.40–**29.59**–41.80	5.69	17.78[a]	48.00
LD	72.60–**98.29**–121.00	13.39	8.77[a]	43.00
WD	36.30–**46.80**–55.00	6.52	10.25[a]	39.00
TL	83.60–**113.36**–140.80	16.01	10.39[a]	44.00
PLI	10.00–**13.22**–16.67	0.02	7.45[a]	50.00
DLI	83.33–**86.78**–90.00	0.02	7.45[a]	50.00
P/DLI	11.11–**15.27**–20.00	0.02	6.60[a]	45.00
P/DWI	41.18–**63.44**–90.48	0.11	2.90[a]	53.00

[a] Significantly different from *G. niphandrodes*; $P > |t| = 0.05$.

that of *G. cuneata*, 2.2 versus 1.5. In general appearance, *G. niphandrodes* differs from *G. cuneata* in life (Fig. 1 versus Fig. 5A and 5B; Fig. 11 versus Fig. 12).

The primites and satellites of gamont associations in *G. niphandrodes* differ significantly from those of *G. polymorpha* in all morphological measurements and ratios except total length (Table 2). In nuclear diameter, *G. polymorpha* is smaller than *G. niphandrodes*, 19.2 versus 35.5 Nuclear placement in the associated gamonts of *G. polymorpha* is uniform, mesad in the anterior third of the deutomerite. Nuclear placement in associated gamonts of *G. niphandrodes* varies, usually off-center in the anterior third of the deutomerite in primites, usually off-center in the posterior third of the deutomerite in satellites. *Gregarina niphandrodes* further differentiates from *G. polymorpha* by pellicular thickness (1.8 versus 2.2); however, the ectocyte is comparable in both species. The general appearance of *G. niphandrodes* differentiates it from *G. polymorpha* in life (Fig. 1 versus Fig. 6; Fig. 11 versus Fig. 13).

The primites and satellites of *G. niphandrodes* differ significantly from those of *G. steini* in all morphological measurements and ratios except primite WP/WD ratio (Table 3). The nuclear diameter of *G. steini* is less than half that of *G. niphandrodes*, 17.6 versus 35.5. Nuclear placement in the associated gamonts of *G. steini* is uniform, off-center in the anterior third of the deutomerite; however, the nucleus is partially obscured by the endocyte. Nuclear placement in associated gamonts of *G. niphandrodes* is variable, usually off-center in the anterior third of the deutomerite in primites, and usually off-center in the posterior third of the deutomerite in satellites. *Gregarina niphandrodes* is differentiated further from *G. steini* by pellicular thickness and appearance of the ectocyte. *Gregarina niphandrodes* has an ectocyte 3.0 thick; there is no apparent ectocyte in fully mature specimens of *G. steini*. The pellicle of *G. niphandrodes* is thicker than that of *G. steini*, 2.2 versus 1.5. The general appearance of *G. niphandrodes* differentiates it from *G. steini* in life (Fig. 1 versus Fig. 7A–C, Fig. 11 versus Fig. 14).

Generic characters define oocysts of *Gregarina* as doliform, navicular, or spherical, and imply no departure in three-dimensional form [7, 13, 19–21, 34]. Oocysts of *G. niphandrodes* display their dorsoventrad flattening and differential pleural aspect only in glycerin preparations that allow free rotation of the oocyst chain. In simple water or gut-fluid preparations, oocyst rotation is restricted, rendering the oocysts doliform or spherical in appearance. *Gregarina niphandrodes, G. cuneata, G. polymorpha* and *G. steini* are all differentiated by unique three-dimensional oocyst morphologies. The oocysts of *G. steini* are reported here for the first time. The oocysts of *G. cuneata* and *G. polymorpha* are redescribed to include their three-dimensional morphology.

Gregarina cuneata

Oocysts (Fig. 8A, B, 16). Dorsum doliform with angles approximately squared by enclosing sheath, length 4.9, width 3.9, rectangular concavity apparent; in pleurad aspect with dorsum surface deeply concave (depth 1.0), venter slightly concave (depth 0.5), pleuron height 2.9; very uniform in size and shape.

Gregarina polymorpha

Oocysts (Fig. 9A, B, 17). Dorsum doliform with angles approximately squared by enclosing sheath, length 4.9, width 3.9, rectangular concavity apparent; in pleurad aspect, dorsum deeply concave (depth 1.0), venter crenulate with mesad and terminal lobes, pleuron height 2.9; very uniform in size and shape.

Gregarina steini

Oocysts (Fig. 10A, B, 18). Dorsum spherical with enclosing sheath, diameter 4.9, elliptical concavity apparent; in pleurad

Table 4. Comparisons of measurements and ratios of fresh and fixed specimens of *Gregarina niphandrodes* n. sp.

Measurment or ratio	t_{obs}	Degrees of freedom
LPI	5.75	145.00[a]
LDI	5.75	145.00[a]
P/DLI	5.68	134.00[a]
LP	4.21	124.00[a]
WP	7.83	119.00[a]
LD	0.12	118.00
WD	1.53	125.00
TL	1.07	115.00

[a] Fresh and fixed specimens significantly different, $P = 0.05$.

aspect, elliptical with dorsad scallop (depth 0.9), pleuron height 2.9; very uniform in size and shape.

DISCUSSION

Gregarine morphology often appears simple and inconclusive under light microscopy; however, these morphological characters are real and distinct, and have remained so over time and space. The gregarines that infect *Tenebrio molitor* are among the most studied assemblage of any insect host and have been subject to numerous descriptions, redescriptions, and reviews at the generic and species level [1, 3, 6, 8, 9, 11–13, 15–17, 19, 22, 25, 29, 31, 34]. Despite name changes and species differentiations, the visual images that characterize these animals have remained consistent in the literature, but our conceptual understanding of the pictures has changed. The original plates of Berndt [1] and Watson [34] are as applicable and recognizable today as they were in Berlin, Germany and Urbana, Illinois almost 90 years ago. Given the enormous global traffic and transport of grain and stored grain products, it is not surprising that as pests of stored grain have become cosmopolitan in their distribution: their parasite assemblages have become cosmopolitan as well.

Previous researchers described gregarine species based on type hosts, epimerite shape, and measurements and ratios of trophozoites and gamonts; however, fixed and embedded protozoan specimens often possess distortion artifacts. Recent researchers place emphasis on gacyst and/or oocyst gametocyst morphology and development, as well as differences between fresh and fixed specimens [14, 26]. Measurements and ratios of fresh and fixed specimens were compared using Student's t-test calculated for unequal variances. In all comparisons, the results of an initial F-test were sufficient to reject the null hypothesis of equal variance between fresh and fixed data sets (Table 4). These comparisons suggest that morphological measurements and ratios are labile over the fixation, dehydration, clearing, and embedding process. While permanent type specimens are essential to insure the stability of a gregarine description, they serve best as indicators of the general form and appearance. Morphological measurements and ratios must be taken from life and preserved in the literature.

The species of *Gregarina* infecting *T. molitor* produces oocysts with a distinct, consistent three-dimensional morphology (Fig. 15–18). Although the gregarine fauna of *T. molitor* are readily differentiated based on gamont morphology, their discrete oocyst morphologies help to confirm the species designations. In other assemblages, prudent use of oocyst morphology may separate gregarine species with similar gamont forms. In experimental infection protocols, oocyst morphology provides a positive identification of infective material.

It is surprising that *G. niphandrodes* has remained unknown until now. However, given the gregarine assemblage diversity of larval *T. molitor*, it is not surprising that most of the work on the gregarines of *T. molitor* has been conducted on larvae, almost to the exclusion of adult hosts. *Gregarina niphandrodes* is evidently restricted to adult forms of *T. molitor* while *G. polymorpha* and *G. steini* appear to be restricted to larval forms [1, 6, 8, 9, 11, 15, 17, 22, 25, 29, 31, 34]. Only *G. cuneata* is found in both host life-cycle stages, though only rarely in the adult [1, 3, 6, 8, 9, 11, 12, 15–17, 22, 25, 29, 31, 34]. These restrictions are described in the literature and evident in host colonies at the University of Nebraska. Anecdotally, we have dissected literally hundreds of adult and larval *T. molitor* from 12 laboratory colonies and have never found *G. niphandrodes* in a larval host. *Gregarina cuneata* is a rare parasite in adult *T. molitor*, but always appears at very low levels never in association, and is easily differentiated from all stages of *G. niphandrodes*. *Gregarina cuneata, G. polymorpha,* and *G. steini* are frequent parasites of larvae, often forming an assemblage within a single animal. *Gregarina polymorpha* and *G. steini* have never been found in an adult host. This apparent restriction within the host life cycle suggests that the gregarine complex in *T. molitor* displays a range of life-cycle stage specificities within a single holometabolic host.

Terminology used in this paper is consistent with Levine [18] with the following clarification: we have used "trophozoite" to indicate an individual that is no longer attached to the gut epithelium of the host, but that has not formed an association. "Gamont" indicates an individual that has formed an association, regardless of relative position within the association or attachment to the host gut epithelium. "Primite" and "satellite" have been reserved for the gamonts of mature associations (separate from the epithelium, with no apparent epimerite). Pairing is precocious in *G. niphandrodes* and no evidence suggests that forming an association guarantees the reproductive fate or success of an individual. In addition, the epimerite is retained, in some form at least, late into development and after separation from the host epithelium. In *G. niphandrodes*, the state of the epimerite does not appear to be a reliable indication of age or development. There is no apparent septum between epimerite and protomerite; however, epimerite, rather than mucron, has been used to reflect the otherwise septate nature of *G. niphandrodes*.

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