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Gregarines from Flour Beetles  
(*Tribolium* spp. and *Palorus*  
*subdepressus*: Coleoptera,  
Tenebrionidae)

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## NEW AND EMENDED DESCRIPTIONS OF GREGARINES FROM FLOUR BEETLES (*TRIBOLIUM* SPP. AND *PALORUS SUBDEPRESSUS*: COLEOPTERA, TENEBRIONIDAE)

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**ABSTRACT:** The following new gregarine taxa are described from larvae of flour beetles (Coleoptera: Tenebrionidae): *Awryregarina billmani*, n. gen., n. sp., from *Tribolium brevicornis*; *Gregarina cloptoni*, n. sp., from *Tribolium freemani*; *Gregarina confusa*, n. sp., from *Tribolium confusum*; and *Gregarina palori*, n. sp., from *Palorus subdepressus*. In addition, the description of *Gregarina minuta* Ishii, 1914, from *Tribolium castaneum*, is emended. Scanning electron micrograph studies of these species' oocysts reveal differences in surface architecture. The *Gregarina* species have oocysts with longitudinal ridges, visible with SEM, whereas *Awryregarina billmani* oocysts have fine circumferential striations; surface architecture is the main feature distinguishing the 2 gregarine genera. Although parasites from adult beetles are not included in the descriptions, adults of all host species can be infected experimentally using oocysts from the new taxa.

The so-called “flour beetles” include a number of stored product pests, particularly species of *Tribolium* and 2 species of *Palorus* (Coleoptera: Tenebrionidae). These insects have been used in ecological studies because of their ease of culture, as well as in research on parasite-induced host behaviors because of their role as intermediate hosts for helminths. Moreover, they have been studied in efforts to develop control methods (Shostak and Smyth, 1998; Yan et al., 1998; Pai and Yan, 2003; Arnaud et al., 2005). Flour beetles also represent interesting host–parasite systems in that all or most of the species, including both adults and larvae, are infected with gregarine parasites (Apicomplexa: Conoidasida: Gregarinasina: Eugregarinorida), and the presumed relationship among hosts provides a tractable laboratory model to explore experimentally the evolutionary constraints on host specificity. Such exploration requires, however, taxonomic description of the parasites involved.

With a group of related hosts and their congeneric or congeneric parasites, molecular methods, used in conjunction morphological characters, allow researchers to develop phylogenetic hypotheses and propose causal explanations such as co-speciation and host capture (Snyder and Tkach, 2001; León-Régagnon and Brooks, 2003). Ideally, however, one should do experimental cross-infections to determine whether host–parasite associations are established by true host–parasite incompatibility or by ecological factors. With most systems involving parasites of vertebrate hosts, logistical burdens make this kind of experimental work very difficult, especially so, when the species are not routinely reared in captivity. With insects such as flour beetles, much of this logistical burden is lifted, especially because their gregarine infections are often as easily manipulated as their hosts (Clopton et al., 1991, 1992; Watwood et al., 1997).

In the case of *Tribolium* species, a number of gregarines have been described already, but the number of host species involved is low, and the descriptions themselves are sometimes not consistent with current practice (see Clopton, 1999), particularly with respect to oocyst morphology (cf. Ghose et al., 1986). The present study was intended to resolve some of the taxonomic problems surrounding the parasites of flour beetles and to bring the gregarine descriptions up to the standards established by

Clopton (1999) in anticipation of using both hosts and parasites in experimental parasite population and host-specificity studies.

### MATERIALS AND METHODS

Insects were obtained from a variety of sources. *Tribolium confusum* and *Tribolium castaneum* were originally collected at the University of Nebraska Poultry Science Feed Mill, Lincoln, Nebraska, during the middle 1980s and have been maintained in culture since (Watwood et al., 1997). *Tribolium madens*, *Tr. brevicornis*, *Tr. freemani*, and *Palorus subdepressus* were obtained from the USDA Post-Harvest Grain Marketing Laboratory, Kansas State University, Manhattan, Kansas, in January 1999. Stock cultures were maintained in plastic jars or conical centrifuge tubes with finely perforated screw-cap lids in media consisting of 45% commercial whole-wheat flour, 45% wheat bran, 7% commercial wheat germ, and 3% baker's yeast by volume. New cultures were started biweekly using 20 adults or mixtures of adults and larvae. Cultures were maintained in incubators set at 27 C and provided with a pan of water inside to maintain humidity.

Hosts were dissected, and both fresh preparations and stained smears were made from guts that had been teased apart by the methods of Kula and Clopton (1999). Fresh preparations were made in *Tenebrio* sp. saline (Belton and Grundfest, 1962). Air-dried smears were fixed in alcohol–formalin–acetic acid, washed in 70% ethyl alcohol, stained in Semichon's acetocarmine, dehydrated, cleared in xylene, and mounted with Canada balsam (Sigma, St. Louis, Missouri) or Damar balsam (Spectrum, Gardena, California) (Pritchard and Kruse, 1982; Kula and Clopton, 1999). For measurements of living organisms, images were taken with a Nikon Coolpix 995 camera fitted with an Optem 25-70-14 adapter (Optem International, Fairport, New York; [www.optemintl.com](http://www.optemintl.com)). Digital photograph numbers were recorded, and the actual total length of each specimen was measured using a calibrated ocular micrometer. Measurements were thus associated with each photograph and used to calibrate measurements taken with image analysis software.

Measurements were taken using Scion Image Beta 4.02 for Windows (Scion Corporation, Frederick, Maryland; [www.scioncorp.com](http://www.scioncorp.com)). For measurements, each photograph was converted to a 15.2-cm-wide (proportions constrained), 100 pixels/inch bitmap file, and the image was adjusted slightly for contrast if necessary, all using Adobe Photoshop 6.0 (Adobe, San Jose, California). Measurements taken were those of the extended gregarine set of Clopton (1999); they were copied to an Excel (Microsoft, Redmond, Washington) spreadsheet, then converted to micrometers using a calibration factor calculated from total length measured with the ocular micrometer and total length in image analysis software units. Some parasite dimensions, however, were simply calculated from those measurements taken instead of being independently measured (the Clopton, 1999, set contains several that allow this approach). The measurements included in the current descriptions are those in Table I. Correlations also were calculated between deutomerite length and nuclear distance posterior to septum. Shape terminology is that recommended by Clopton (2004a).

Gametocysts were harvested by isolating larvae overnight in the outside moat of Falcon 3010 plastic, organ tissue-culture dishes with a small piece of wet paper towel in the center well and the cover added. Gametocysts were then picked up with a fine camel hairbrush and trans-

TABLE I. Morphological characters measured and abbreviations for these characters as used in this article.

Abbreviation	Character
TL	Total length
PL	Protomerite length
PLA	Protomerite length anterior to widest point
PLP	Protomerite length posterior to widest point
PWM	Maximum protomerite width
PDSW	Width of the protomerite-deutomerite septum
DL	Deutomerite length
DWE	Deutomerite width at midpoint of deutomerite
DWM	Maximum deutomerite width
DLA	Length of deutomerite anterior to widest point
DLP	Length of deutomerite posterior to widest point
ND	Greatest nuclear dimension
NDP	Distance of nucleus posterior to septum
$r$ DL:NDP; $p/r = 0/$	Correlation between deutomerite length and NDP
$r$ DWM:NDP; $p/r = 0/$	Correlation between maximum deutomerite width and NDP
TL/PL	Total length/protomerite length ratio
TL/DL	Total length/deutomerite length ratio
DL/PL	Deutomerite/protomerite length ratio
PL/PWM	Protomerite length/maximum width ratio
PL/PLA	Protomerite length/length anterior to widest point ratio
PL/PDSW	Protomerite length/septum width ratio
PWM/PDSW	Protomerite maximum width/septum width ratio
DL/DWE	Deutomerite length/width and mid-point ratio
DL/DWM	Deutomerite length/maximum width ratio
DLA/DL	Deutomerite length anterior to widest point/deutomerite length ratio
DLA/DLP	Deutomerite length anterior to widest point/posterior to widest point ratio
DWM/DWE	Deutomerite maximum width/width at midpoint ratio

ferred to 1% neutral buffered formalin to be washed for a few minutes before isolation for further observation or dehiscence. For gametocyst photography, cysts were placed in a hanging drop of 1:1 glycerin:tap water on a cover glass and allowed to dehisce. The cover glass was then placed on a slide, drop side down, for observation of dehiscence mechanism.

Oocysts were prepared for digital photography by allowing gametocysts to dehisce on 22 × 22-mm cover glasses resting on the center well of covered Falcon plastic organ culture dishes with water in the moat, then inverting the cover glasses on a slide and tacking the corners down with glue. With this technique, gametocysts could complete their early development in a high humidity atmosphere as demonstrated by Clopton and Janovy (1993), and oocysts could be photographed later under oil immersion in their "natural" state, i.e., in air, instead of being suspended in media such as glycerol or agar (Clopton, 1995; Clopton et al., 2004). Measurements were taken with ScionImage software as described above, but calibrations were done using a stage micrometer photograph. Because oocyst chains are twisted in such preparations, at least 50 oocyst lengths were measured, but the width and thickness measurements could only be taken on those cysts oriented in the proper direction. Thus, length, width, and thickness measurements are not necessarily from the same individual oocysts, but from the oocysts in chains resulting from dehiscence.

Oocysts were prepared for scanning electron microscopy in 2 ways. First, oocyst chains were placed on 15-mm round cover glasses coated with poly-L-lysine (Sigma-Aldrich, St. Louis, Missouri) according to package instructions. These cover glasses plus oocysts were then fixed for 2 hr in 3% phosphate buffered glutaraldehyde, pH 7.2, washed in 0.1 M phosphate-buffered sucrose solution, postfixated for 1 hr in 1% osmium tetroxide in 0.1 M phosphate buffer, washed with distilled water, dehydrated through an acetone series, critical point-dried using a Sorvall critical-point drying system (DuPont, Newtown, Connecticut), and sputter-coated with 200 Å gold-palladium using a Technics (Osaka, Japan) Hummer II sputterer. In the second method, oocysts were simply sputter-coated without prior fixation. Scanning electron microscopy was performed at 15 kV with a Hitachi (Tokyo, Japan) 3000N SEM. After

comparison of electron micrographs taken of oocysts prepared by both methods, the latter was chosen for all the SEMs because no detectable differences in surface features could be observed in specimens prepared by the different methods.

Parasite hapantotypes and paratypes were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska State Museum (UNSM), Lincoln, Nebraska. Host symbiotypes were prepared according to instructions provided by Dr. Brett Ratcliffe, Curator of Entomology, UNSM, and deposited in that facility. Accession numbers for both hosts and parasites are provided in the taxonomic sections.

Drawings were made either with the aid of a camera lucida or by tracing video screen images on acetate sheets then tracing those images on to velum. Statistical analysis was performed with Excel and Statview. All measurements are reported in micrometers as the mean (range, standard deviation, n).

## DESCRIPTION

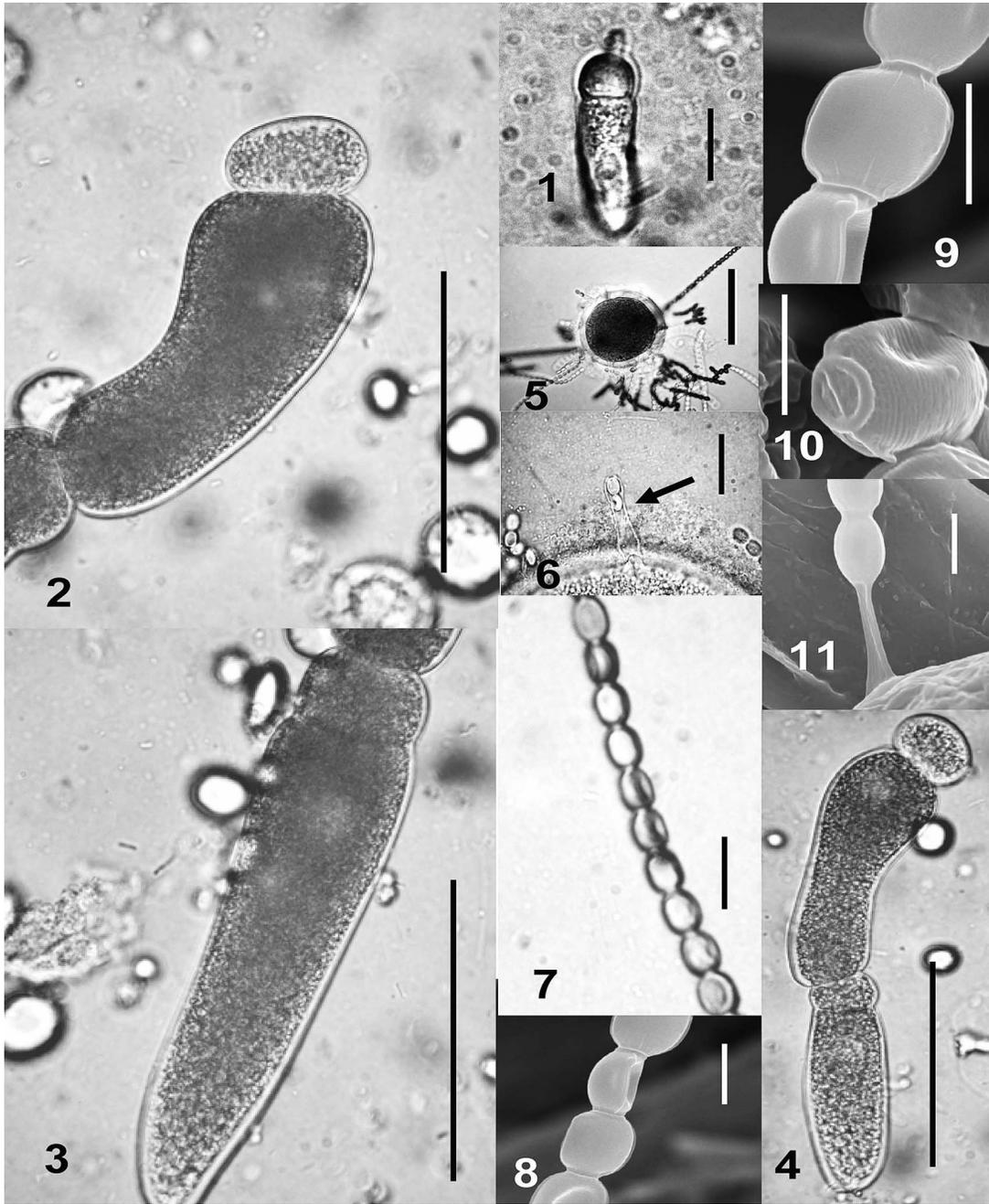
### *Awryregarina* g. nov.

*Diagnosis:* Eugregarinorida Léger, 1892, sensu Clopton (2000); Septatorina Lankester, 1885, sensu Clopton (2000), Gregarinicae Chakravarty, 1960, sensu Clopton (2000), Gregarinidae Labbé, 1899, sensu Clopton (2000); with characters of *Awryregarina* g. nov.: epimerite simple short cone; association precocious, cephalocaudal, biassociative; gamonts anisogamous, primate with protomerite conspicuously offset laterally ("awry"), mature satellite longer than primate and with attenuated posterior end; gametocysts spherical, dehiscing in chains through sporoducts; oocysts approximately spherical with fine circumferential striations.

### Taxonomic summary

*Type species:* *Awryregarina billmani* sp. nov.

*Etymology:* The generic name *Awryregarina* is derived from the consistently offset protomerite of the mature primate, giving the ap-



FIGURES 1–11. *Awrygregarina billmani*, n. sp. in various stages. (1) Young trophozoite with epimerite. Bar = 10  $\mu$ m. (2–3) Primitive and satellite of associated pair, respectively. Bar = 100  $\mu$ m. (4) Smaller associated pair. Bar = 50  $\mu$ m. (5) Gametocyst in dehiscence (in glycerin). Bar = 100  $\mu$ m. (6) Oocyst tube (arrow) on gametocyst; oocysts allow estimation of tube length. Bar = 20  $\mu$ m. (7) Oocyst chain. Bar = 10  $\mu$ m. (8–10) Oocysts (SEM) showing ends and fine circumferential striations. Bar = 5  $\mu$ m. (11) Oocysts showing what appear to be an external sheath binding them into a chain.

pearance of the compartment being awry or askew in comparison to typical species of *Gregarina*.

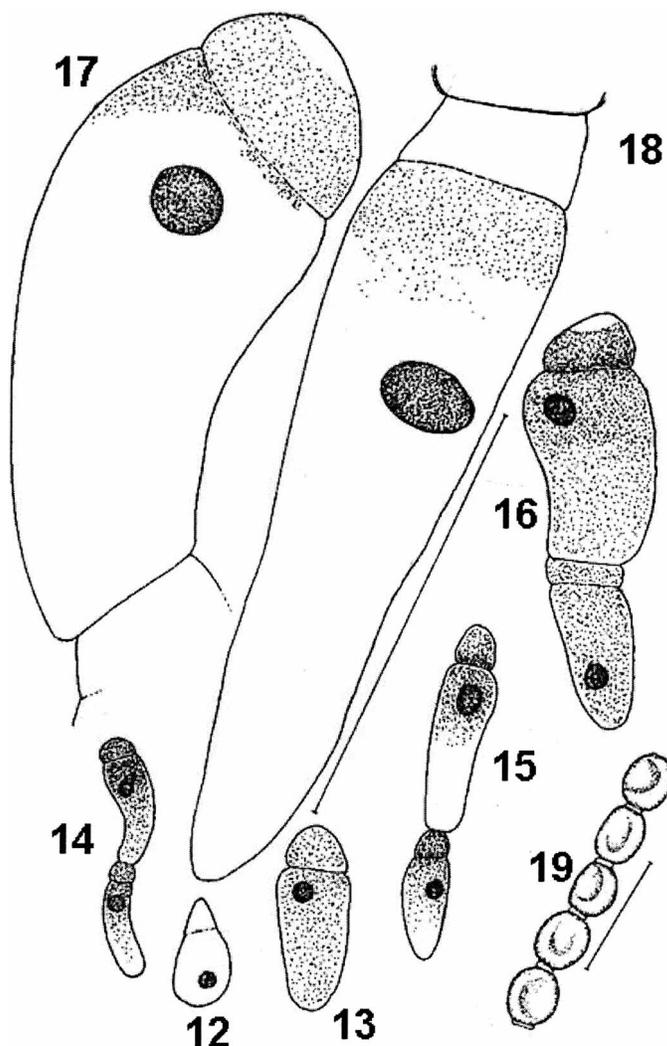
**Remarks**

Dehiscence in chains through sporoducts, precocious association, and cephalocaudal association, all consistent with cardinal characters of the Gregarinidae. Oocyst ultrastructural characters, especially nature of fine surface striations, clearly distinguish this genus from those currently considered species of *Gregarina* and for which SEM-level oocyst characters are available. Major distinguishing feature of this genus, sepa-

rating it from *Gregarina*, is oocyst surface architecture as revealed by SEM. Series of low ridges encircling oocyst parallel to equator is in stark contrast to high longitudinal ridges characteristic of *Gregarina* species from congeneric hosts.

***Awrygregarina billmani* n. sp.**  
(Figs. 1–19)

*Trophozoite* (Figs. 1, 12–13): Developing trophozoites attached to host epithelium; primitives and satellites distinguishable at all developmental stages by offset protomerite (primitive) and attenuated posterior



FIGURES 12–19. Line drawings of *Awrygregarina billmani*, n. sp. (12–13) Young trophozoites. (14–16) Paired gamonts in various stages of maturity. (17) Mature associated primitive. (18) Mature associated satellite. (19) Oocysts. Figures 12–18, bar = 100  $\mu$ m; Figure 19, bar = 10  $\mu$ m.

end (satellite); epimerite papillalike, visible usually only on smallest trophozoites. Measurements and correlations between measurements are listed in Table II.

**Gamonts and association** (Figs. 2–4, 14–18): Primitive: protomerite ovoid to broadly ovoid, tilted away from primitive axis; deutomerite oblong to narrowly oblong. Satellite: protomerite ovoid to broadly ovoid; deutomerite obovoid to narrowly obovoid. Measurements and correlations between measurements in Table II.

**Gametocysts** (Figs. 5–6): Spherical to oval, white translucent when shed, with obvious sporoducts when allowed to dehisce in 1:1 glycerin: water; 119.1 (95.0–142.5, 11.3, 15) by 102.6 (85.5–123.5, 10.9, 15).

**Oocysts** (Figs. 7–11, 19): Broadly dolioform, in chains, often depressed or somewhat concave on 1 surface; length 4.8 (3.7–5.5, 0.4, 50), width 3.5 (2.9  $\times$  4.1, 0.3, 50), thickness 2.2 (1.6–2.8, 0.2, 50). Ultrastructurally with a coating, presumably of gametocyst origin, over oocyst chain, series of low ridges or fine striations parallel to equator, and raised ring at each end of each oocyst (Figs. 8–10).

#### Taxonomic summary

**Host:** *Tribolium brevicornis* LeConte, 1859.

**Symbiotype:** University of Nebraska State Museum, Entomology Division collection, vouchers: J. Janovy, Jr. 001–010 (10 pinned adults on points); J. Janovy, Jr., 050 (larvae in vial).

**Host records:** *Tribolium brevicornis* LeConte, 1859; larvae; laboratory infections.

**Locality:** University of Nebraska Lincoln; School of Biological Sciences, Manter Hall, Lincoln, Nebraska, cultures.

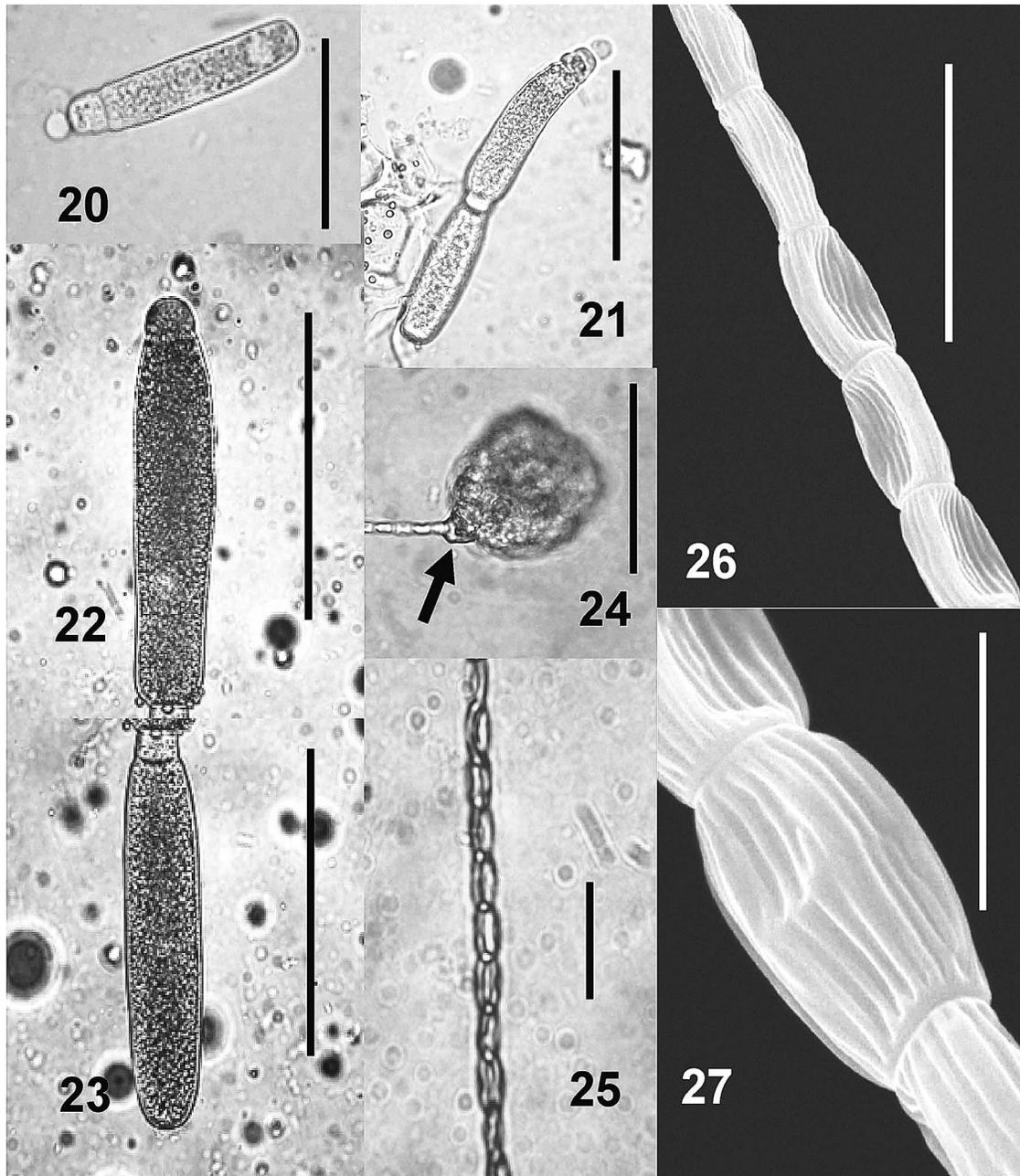
**Infection site:** Midgut.

**Specimens:** University of Nebraska State Museum, Harold W. Manter Laboratory of Parasitology collections; HWML48434 (hapantotype); HWML48435–HWML48438 (paratypes).

**Etymology:** The new species is named in honor of Dr. James K. Billman, Jr., a generous supporter of University of Nebraska undergraduate research and teaching, especially at the Cedar Point Biological Station.

#### Remarks

“Trophozoites” include individuals that for some reason remain unassociated, thus this stage is designated rather arbitrarily, given that some are obviously very tiny and others are very large. In the case of *Awrygregarina billmani*, presumptive primitives and satellites have morphological features that allow one to distinguish the 2 mating types while they are still very small, and the 2 types retain characteristic morphology even if they remain singlets until they grow relatively large. Epimerites are visible only on the very small ones; however, designation of these individuals as “immature” is arbitrary because epimerites can be lost in dissection or in the process of making permanent slides from smears. Minimum pairing size is one potentially useful criterion for distinguishing immature from mature trophozoites, although the term “precocious” as applied to pairing, and used as a taxonomic character,



FIGURES 20–27. *Gregarina cloptoni*, n. sp., various stages. (20) Young trophozoite. Bar = 50  $\mu$ m. (21) Immature associated pair, showing epimerite. Bar = 50  $\mu$ m. (22–23) Mature primite and satellite, respectively. Bar = 100  $\mu$ m. (24) Gametocyst showing sporoduct (arrow). Bar = 100  $\mu$ m. (25) Oocysts. Bar = 10  $\mu$ m. (26–27) SEMs of oocysts showing longitudinal ridges (Fig. 26, bar = 10  $\mu$ m; Fig. 27, bar = 5  $\mu$ m).

tends to call this criterion into question. It is obvious from the literature that gregarine mating behavior is quite diverse, but generally falls into 2 categories, i.e., late and precocious. *Awrygregarina billmani* pairs precociously, but some individuals do not find mates. Failure to pair does not inhibit growth because singlets can grow as large as their mated co-symbionts.

***Gregarina cloptoni* n. sp.**  
(Figs. 20–33)

*Trophozoite* (Figs. 20, 28–29): Epimerite globular. Protomerite shallow to broadly ovoid; deutomerite narrow to very narrowly oblong.

Measurements and correlations between measurements are given in Table II.

*Gamonts and association* (Figs. 21–23, 31–32): Primite: TL 111.1 (46.0–214.0, 37.0, 68); protomerite very shallow to broadly ovoid; deutomerite narrow to very narrowly oblong. Satellite: protomerite depressed to shallowly ovoid; deutomerite narrow to very narrowly oblong. Measurements and correlations between measurements in Table II.

*Gametocysts* (Fig. 24): Translucent and white when shed, with hyaline space up to 10  $\mu$  wide surrounding inner cytoplasmic mass; subspherical, 94.4 (85.5–104.5, 5.6, 15) by 87.4 (76.0–95.0, 6.2, 15).

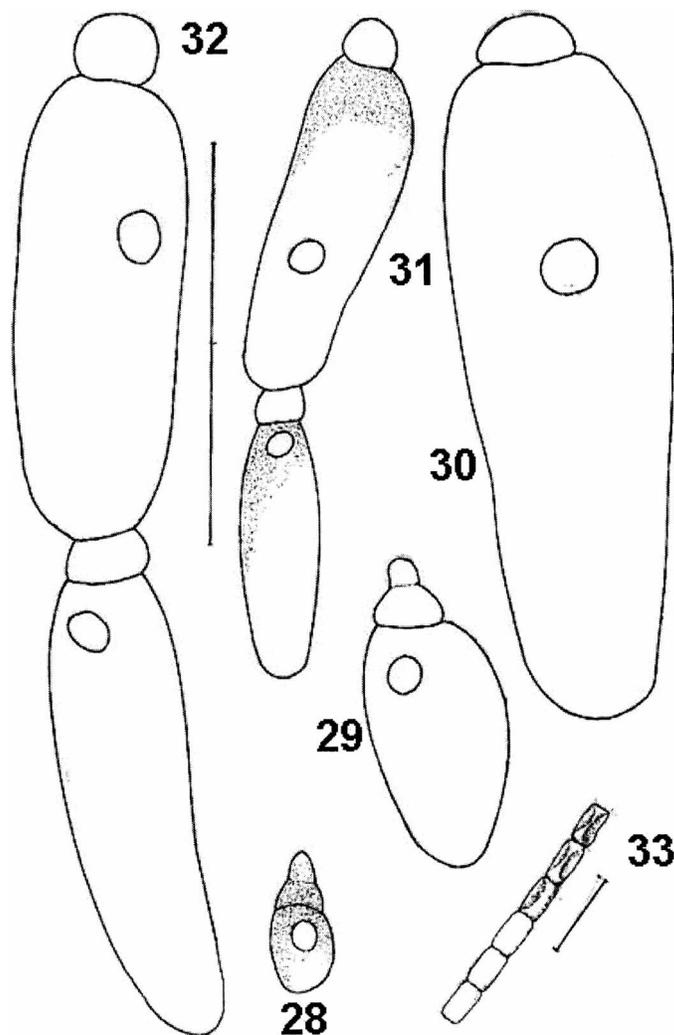
*Oocysts* (Figs. 25–27, 33): In chains, end-to-end with long axis par-

TABLE II. Measurements of morphological characters for 4 new species of *Gregarina* and for the emended description of *G. minuta*. All measurements are in micrometers and reported as mean (range, standard deviation).

Stage and character	<i>Awrygregarina billmani</i>	<i>Gregarina cloptoni</i>	<i>Gregarina confusa</i>	<i>Gregarina palori</i>	<i>Gregarina minuta</i>
Trophozoite; n =	28	82	41	33	43
TL	113.6 (7.1–275.0, 91.0)	82.9 (18.8–211.0, 43.4)	97.5 (46.0–154.0, 70.0)	99.3 (22.1–196.0, 47.2)	80.3 (18.0–180.0, 41.5)
PL	18.7 (1.7–50.8, 14.4)	10.5 (4.8–17.5, 2.7)	10.9 (9.7–11.6, 2.7)	11.5 (5.3–20.0, 3.3)	10.1 (4.1–16.6, 3.0)
PLA	12.7 (1.2–30.6, 9.4)	7.5 (3.8–15.0, 2.3)	8.6 (7.1–9.2, 1.4)	8.8 (2.6–15.2, 2.3)	7.3 (3.6–12.8, 2.3)
PLP	6.0 (0.2–20.2, 6.1)	3.0 (0.0–7.5, 1.6)	2.4 (0.4–6.2, 1.2)	2.7 (0.3–5.0, 1.3)	2.7 (0.4–5.5, 1.2)
PWM	24.1 (2.0–66.2, 18.7)	12.1 (6.7–26.5, 3.7)	12.4 (8.3–13.6, 3.8)	14.6 (6.1–28.9, 5.0)	12.0 (6.4–20.6, 3.7)
PDSW	21.8 (2.3–61.1, 16.3)	12.1 (6.7–26.5, 3.7)	12.8 (7.5–13.6, 4.8)	13.7 (5.3–25.1, 4.8)	11.8 (6.1–20.1, 3.7)
DL	94.9 (5.4–239.6, 77.4)	72.5 (14.0–197.7, 41.2)	86.6 (18.7–165.0, 43.2)	87.8 (16.9–181.2, 44.4)	70.2 (13.9–163.4, 39.1)
DWE	27.6 (1.9–86.6, 24.4)	17.9 (8.8–42.3, 8.1)	18.8 (10.5–17.4, 8.0)	21.6 (10.4–44.6, 9.8)	16.3 (6.6–37.0, 7.1)
DWM	30.0 (2.0–93.5, 25.0)	18.5 (8.8–42.3, 8.3)	18.3 (9.9–19.4, 6.0)	21.4 (8.6–43.1, 9.5)	16.5 (6.6–38.4, 7.4)
DLA	11.3 (0.8–36.8, 9.4)	18.7 (2.6–83.8, 16.7)	14.2 (4.8–46.2, 11.2)	25.8 (5.5–90.0, 22.4)	21.5 (3.1–115.0, 26.7)
DLP	83.6 (4.5–226.8, 70.2)	53.7 (5.5–188.4, 34.0)	72.4 (16.3–144.9, 40.0)	62.1 (8.1–171.6, 45.5)	48.7 (10.8–124.9, 31.4)
NDP	9.6 (1.4–20.4, 5.9)	7.5 (1.5–14.5, 2.3)	8.9 (6.3–11.9, 2.6)	8.4 (2.3–16.9, 3.1)	7.6 (3.3–13.0, 2.6)
NDP	13.2 (1.9–45.9, 12.1)	25.3 (2.6–133.3, 25.1)	35.5 (2.7–61.5, 11.7)	37.1 (2.0–133.6, 32.2)	31.8 (2.6–96.0, 28.4)
<i>r</i> DL:NDP; <i>p/r</i> = 0/	0.60; <0.01	0.67; <0.01	0.58; <0.01	0.45; <0.01	0.78; <0.01
<i>r</i> DWM:NDP; <i>p/r</i> = 0/	0.75; <0.01	0.51; <0.01	0.49; <0.01	0.58; <0.01	0.62; <0.01
TL/PL	5.7 (3.2–8.7, 1.5)	7.6 (3.4–15.9, 2.5)	8.7 (3.8–14.7, 2.9)	8.3 (4.2–13.2, 2.4)	7.6 (4.3–12.5, 2.5)
TL/DL	1.2 (1.1–1.5, 0.1)	1.2 (1.1–1.4, 0.1)	1.2 (1.1–1.4, 0.1)	1.2 (1.1–1.3, 0.1)	1.2 (1.1–1.3, 0.1)
DL/PL	4.7 (2.2–7.7, 1.5)	6.6 (2.4–14.9, 2.5)	7.7 (2.8–13.7, 2.9)	7.3 (3.2–12.2, 2.4)	6.6 (3.3–11.5, 2.5)
PL/PWM	1.5 (0.1–2.1, 0.2)	0.8 (0.6–1.4, 0.1)	0.9 (0.6–1.3, 0.2)	0.8 (0.6–1.2, 0.1)	0.9 (0.5–1.6, 0.2)
PL/PLA	1.5 (1.0–2.1, 0.3)	1.4 (0.7–2.5, 0.3)	1.3 (1.1–1.7, 0.2)	1.4 (1.1–2.3, 0.3)	1.4 (1.1–1.8, 0.2)
PL/PDSW	0.9 (0.5–1.4, 0.3)	0.9 (0.5–1.2, 0.2)	0.9 (0.4–1.4, 0.2)	0.9 (0.6–1.3, 0.2)	0.9 (0.6–1.7, 0.2)
PWM/PDSW	1.1 (0.8–1.4, 0.2)	1.0 (0.8–1.3, 0.1)	1.0 (0.8–1.4, 0.1)	1.1 (0.8–1.3, 0.1)	1.0 (0.8–1.2, 0.1)
DL/DWE	3.4 (1.4–6.4, 1.2)	4.0 (1.6–8.3, 1.3)	4.6 (2.0–9.4, 1.7)	4.0 (1.6–6.6, 1.2)	4.2 (1.5–8.8, 1.4)
DL/DWM	3.1 (1.4–5.4, 0.9)	3.9 (1.6–9.1, 1.3)	8.3 (1.6–37.0, 6.1)	4.1 (4.6–7.2, 1.1)	4.1 (1.5–9.1, 1.3)
DLA/DL	0.2 (0.1–0.4, 0.1)	0.3 (0.1–0.7, 0.2)	0.2 (0.1–0.6, 0.1)	0.3 (0.1–0.8, 0.2)	0.3 (0.1–0.9, 0.2)
DLA/DLP	0.2 (0.1–0.6, 0.1)	0.5 (0.1–2.7, 0.5)	0.3 (0.1–1.7, 0.3)	0.7 (0.1–3.6, 0.7)	0.7 (0.1–7.0, 0.2)
DWM/DWE	1.1 (0.9–1.5, 0.1)	1.0 (0.9–1.4, 0.1)	1.0 (0.8–1.2, 0.1)	1.0 (0.8–1.2, 0.1)	1.0 (0.8–1.3, 0.1)
Gamont–primitive; n =	76	68	94	57	56
TL	104.9 (31.8–236.3, 43.2)	111.1 (46.0–214.0, 37.0)	104.3 (42.5–179.9, 33.5)	116.6 (52.1–228.8, 40.1)	113.0 (58.3–191.7, 29.8)
PL	18.3 (5.3–43.7, 6.6)	12.5 (9.0–19.6, 2.4)	11.9 (6.4–21.1, 2.6)	13.2 (9.1–22.5, 3.2)	12.5 (8.3–21.2, 2.3)
PLA	12.5 (4.1–24.6, 4.2)	9.2 (5.0–15.9, 1.9)	9.1 (4.7–16.4, 2.3)	10.1 (4.1–19.4, 2.7)	9.2 (4.6–13.4, 1.6)
PLP	5.8 (0.5–19.7, 3.1)	3.4 (0.0–8.5, 1.6)	2.8 (0.0–5.2, 1.2)	3.2 (0.6–7.8, 1.8)	3.4 (0.2–7.8, 1.5)
PWM	28.6 (7.7–58.1, 12.1)	14.5 (8.8–24.7, 3.2)	14.5 (7.5–23.0, 3.2)	17.0 (9.2–24.0, 3.7)	14.1 (8.2–23.4, 3.1)
PDSW	22.5 (6.7–54.2, 9.9)	13.9 (8.8–22.2, 3.2)	14.5 (8.0–23.1, 3.1)	16.1 (8.6–24.0, 3.9)	14.1 (8.9–25.8, 3.3)
DL	86.6 (25.3–192.6, 37.1)	98.6 (34.2–204.0, 35.8)	92.5 (36.1–163.0, 31.7)	103.4 (42.1–210.4, 37.9)	100.4 (49.2–177.7, 28.8)
DWE	26.9 (8.6–69.8, 14.5)	19.4 (10.9–40.0, 5.3)	21.3 (10.9–51.3, 7.0)	27.7 (10.7–54.5, 10.7)	19.6 (9.6–44.0, 7.8)
DWM	33.0 (8.8–70.0, 16.2)	20.5 (11.5–40.0, 5.5)	21.9 (9.7–46.5, 6.7)	28.7 (10.4–52.2, 10.9)	20.9 (10.4–45.9, 8.1)
DLA	14.9 (1.3–96.7, 15.0)	28.4 (6.4–110.0, 27.6)	15.0 (3.8–124.7, 17.1)	32.7 (7.4–112.2, 23.6)	32.8 (8.0–114.9, 28.6)
DLP	71.7 (20.4–162.1, 31.1)	70.3 (11.4–155.6, 37.7)	77.4 (18.9–136.3, 28.7)	70.6 (15.3–163.3, 31.6)	67.6 (8.0–137.2, 31.6)
ND	11.4 (3.6–22.0, 4.2)	9.0 (5.5–15.1, 2.0)	9.2 (4.7–18.6, 2.4)	10.0 (5.4–14.8, 2.4)	9.1 (5.7–16.1, 2.1)
NDP	21.4 (3.6–97.7, 20.5)	45.3 (2.1–122.4, 32.7)	35.1 (2.7–115.9, 28.7)	51.2 (3.1–132.5, 31.9)	38.9 (3.3–102.2, 26.8)
<i>r</i> DL:NDP; <i>p/r</i> = 0/	0.40; <0.01	0.71; <0.01	0.60; <0.01	0.58; <0.01	0.42; <0.01
<i>r</i> DWM:NDP; <i>p/r</i> = 0/	0.29; <0.05	0.61; <0.01	0.39; <0.01	0.47; <0.01	0.19; > 0.05
TL/PL	5.6 (3.6–9.2, 1.0)	8.9 (3.9–21.4, 2.7)	8.7 (5.0–14.3, 2.0)	8.8 (5.2–15.9, 2.2)	9.1 (5.0–18.2, 2.4)

TABLE II. Continued.

Stage and character	<i>Awyregregarina billmani</i>	<i>Gregarina cloptoni</i>	<i>Gregarina confusa</i>	<i>Gregarina palori</i>	<i>Gregarina minuta</i>
TL/DL	1.2 (1.1-1.4, 0.1)	1.1 (1.0-1.3, 0.1)	1.1 (1.1-1.3, 0.1)	1.1 (1.1-1.2, 0.1)	1.1 (1.1-1.2, 0.1)
DL/PL	4.6 (2.6-8.2, 1.0)	7.9 (2.9-20.4, 2.7)	7.7 (4.0-13.3, 2.0)	7.8 (4.2-14.9, 2.2)	8.1 (4.0-17.2, 2.4)
PL/PWM	1.5 (0.9-2.0, 0.1)	0.9 (0.5-1.2, 0.1)	0.8 (0.5-1.2, 0.2)	0.8 (0.6-1.2, 0.2)	0.9 (0.5-1.4, 0.2)
PL/PLA	1.5 (0.9-2.0, 0.2)	1.4 (1.0-2.0, 0.2)	1.3 (1.0-1.7, 0.2)	1.3 (1.1-2.2, 0.2)	1.4 (1.0-1.8, 0.2)
PL/PDSW	0.9 (0.4-1.4, 0.2)	0.9 (0.6-1.3, 0.2)	0.8 (0.5-1.2, 0.2)	0.8 (0.6-1.3, 0.2)	0.9 (0.4-1.6, 0.2)
PWM/PDSW	1.3 (0.7-1.7, 0.2)	1.0 (0.8-1.3, 0.1)	1.0 (0.8-1.3, 0.1)	1.1 (0.9-1.3, 0.1)	1.0 (0.8-1.3, 0.1)
DL/DWE	3.4 (1.8-6.0, 0.8)	5.1 (1.8-7.8, 1.4)	4.5 (2.4-8.0, 1.2)	3.9 (2.4-6.9, 1.0)	5.4 (3.2-7.8, 1.1)
DL/DWM	2.7 (1.9-5.1, 0.6)	4.8 (1.8-7.6, 1.3)	8.4 (1.2-14.5, 3.1)	3.7 (2.3-6.5, 0.9)	5.0 (3.2-7.3, 1.0)
DLA/DL	0.2 (0.0-0.7, 0.1)	0.3 (0.1-0.9, 0.2)	0.2 (0.1-0.8, 0.1)	0.3 (0.1-0.7, 0.2)	0.3 (0.1-0.9, 0.3)
DLA/DLP	0.2 (0.0-2.6, 0.4)	0.9 (0.1-5.9, 1.5)	0.3 (0.1-5.4, 0.8)	0.6 (0.1-2.3, 0.6)	1.2 (0.1-12.4, 2.2)
DWM/DWE	1.2 (0.9-1.7, 0.2)	1.1 (0.9-1.3, 0.1)	1.0 (0.8-1.4, 0.1)	1.0 (0.9-1.4, 0.1)	1.1 (1.0-1.4, 0.1)
Giant-satellite; n =	76	68	94	57	56
TL	124.2 (20.5-283.3, 72.1)	94.6 (31.3-191.0, 38.3)	100.1 (34.9-216.6, 31.8)	99.9 (42.9-172.8, 34.1)	98.4 (27.3-182.5, 38.5)
PL	17.0 (3.5-44.4, 9.2)	8.3 (3.5-14.0, 2.4)	9.4 (3.4-16.7, 1.6)	10.1 (5.2-18.3, 2.8)	8.5 (3.2-17.0, 2.5)
PLA	11.8 (1.0-36.8, 7.4)	6.1 (1.0-11.6, 2.3)	7.0 (1.9-12.2, 2.7)	7.3 (3.3-15.0, 2.5)	6.0 (1.8-12.0, 1.8)
PLP	5.5 (1.0-14.5, 3.2)	2.2 (0.0-5.4, 1.1)	2.4 (0.1-5.4, 1.3)	2.8 (0.1-6.3, 1.5)	2.4 (0.1-6.1, 1.3)
PWM	25.3 (6.5-64.3, 13.2)	13.1 (7.7-32.5, 3.8)	13.7 (7.5-30.5, 3.1)	16.2 (8.1-23.9, 4.1)	13.3 (6.9-21.9, 3.6)
PDSW	23.6 (5.5-69.6, 13.3)	12.6 (8.1-23.3, 3.0)	13.9 (7.3-23.1, 3.1)	15.8 (6.4-24.9, 4.6)	13.2 (5.9-22.9, 4.1)
DL	107.2 (16.4-248.8, 63.5)	86.4 (27.2-179.2, 36.3)	89.5 (30.4-205.1, 37.9)	89.8 (37.8-154.5, 32.0)	90.0 (21.7-169.9, 36.4)
DWE	25.0 (6.2-72.1, 14.3)	18.8 (8.0-37.0, 5.9)	20.3 (8.8-43.5, 2.6)	25.9 (10.0-51.9, 10.1)	19.7 (8.0-46.2, 8.1)
DWM	27.8 (6.9-78.9, 16.6)	19.1 (8.3-40.3, 6.3)	19.6 (8.2-40.7, 3.7)	25.6 (9.5-49.5, 9.9)	20.0 (8.0-47.1, 8.8)
DLA	11.3 (1.2-47.9, 8.1)	20.2 (5.5-57.6, 11.4)	15.7 (4.8-94.7, 6.9)	21.4 (7.3-61.2, 10.4)	21.4 (3.8-52.5, 10.9)
DLP	95.8 (14.1-233.7, 58.8)	66.2 (15.0-155.4, 34.0)	73.9 (23.6-174.2, 33.1)	68.4 (21.5-127.9, 28.3)	68.6 (16.0-141.2, 30.0)
ND	10.9 (3.0-22.6, 4.9)	8.1 (2.8-14.4, 2.1)	9.0 (4.7-17.3, 0.5)	9.3 (5.5-15.9, 2.0)	8.9 (3.9-15.4, 2.7)
NDP	16.6 (1.2-108.5, 15.6)	31.9 (5.1-146.0, 27.6)	31.1 (3.0-120.1, 30.0)	40.8 (2.0-112.8, 28.0)	33.5 (3.6-131.9, 24.8)
r DL:NDP; p/r = 0/	0.37; <0.01	0.39; <0.01	0.32; <0.01	0.49; <0.01	0.47; <0.01
r DWM:NDP; p/r = 0/	0.32; <0.01	0.26; <0.05	0.20; <0.05	0.39; <0.05	0.45; <0.01
TL/PL	7.2 (3.8-11.1, 1.4)	11.3 (5.7-18.1, 2.5)	10.5 (5.2-18.7, 2.8)	9.9 (5.9-20.4, 2.5)	11.4 (4.8-17.1, 2.4)
TL/DL	1.2 (1.1-1.4, 0.1)	1.1 (1.1-1.2, 0.1)	1.1 (1.1-1.2, 0.1)	1.1 (1.1-1.2, 0.1)	1.1 (1.1-1.3, 0.1)
DL/PL	6.2 (2.8-10.1, 1.4)	10.3 (4.7-17.1, 2.5)	9.5 (4.2-17.7, 2.8)	8.9 (4.9-19.4, 2.5)	10.4 (3.8-16.1, 2.4)
PL/PWM	1.6 (0.5-3.6, 0.1)	0.6 (0.2-1.0, 0.2)	0.7 (0.3-1.1, 0.1)	0.6 (0.3-1.5, 0.2)	0.6 (0.4-1.2, 0.1)
PL/PLA	1.6 (0.5-3.6, 0.4)	1.5 (0.9-4.0, 0.5)	1.4 (1.0-2.1, 0.2)	1.4 (1.0-2.7, 0.3)	1.4 (1.0-2.2, 0.2)
PL/PDSW	0.7 (0.4-2.7, 0.3)	0.7 (0.3-1.0, 0.2)	0.7 (0.4-1.0, 0.1)	0.7 (0.3-1.8, 0.2)	0.7 (0.4-1.5, 0.2)
PWM/PDSW	1.1 (0.8-4.5, 0.4)	1.0 (0.8-2.5, 0.2)	1.0 (0.8-1.4, 0.1)	1.0 (0.9-1.5, 0.1)	1.0 (0.8-2.6, 0.2)
DL/DWE	4.2 (1.8-11.4, 1.3)	4.5 (2.0-6.9, 1.1)	4.5 (2.4-8.1, 1.1)	3.5 (2.2-5.5, 0.7)	4.6 (2.3-6.5, 1.0)
DL/DWM	3.9 (1.9-18.9, 1.9)	4.5 (1.9-7.2, 1.2)	4.6 (2.5-8.5, 1.1)	3.6 (2.2-5.5, 0.7)	4.6 (2.5-6.5, 0.9)
DLA/DL	0.1 (0.1-0.4, 0.1)	0.3 (0.1-0.7, 0.1)	0.2 (0.1-0.6, 0.1)	0.3 (0.1-0.6, 0.1)	0.2 (0.1-0.6, 0.1)
DLA/DLP	0.1 (0.1-0.8, 0.1)	0.4 (0.1-2.8, 0.5)	0.2 (0.1-1.8, 0.2)	0.4 (0.1-1.6, 0.2)	0.4 (0.1-1.4, 0.2)
DWM/DWE	1.1 (0.3-1.9, 0.2)	1.0 (0.9-1.2, 0.1)	1.0 (0.7-1.1, 0.1)	1.0 (0.8-1.1, 0.1)	1.0 (0.8-1.1, 0.1)



FIGURES 28–33. *Gregarina cloptoni*, n. sp. (28–30) Trophozoites of various ages. (31) Immature associated pair. (32) Mature associated pair. (33) Oocysts. Figures 28–32, bar = 100  $\mu$ m; Figure 33, bar = 10  $\mu$ m.

allel to chain axis but with individual cysts rotated at various angles along axis; oblong, irregularly wrinkled, indented, or compressed when dry; length 5.4 (4.6–6.3, 0.4, 50), width 2.9 (2.4–3.5, 0.3, 50), thickness 1.8 (1.2–2.6, 0.3, 50). Ultrastructurally with series of ridges parallel to long axis (Figs. 26–27).

#### Taxonomic summary

*Host*: *Tribolium freemani* Hinton 1949.

*Symbiotype*: University of Nebraska State Museum, Entomology Division collection, vouchers: J. Janovy, Jr., 031–040 (10 pinned adults on points); J. Janovy, Jr., 053 (larvae in vial).

*Host records*: *Tribolium freemani* Hinton 1949 (larvae; laboratory infections).

*Locality*: University of Nebraska Lincoln; School of Biological Sciences, Manter Hall, Lincoln, Nebraska, cultures.

*Infection site*: Midgut.

*Specimens*: University of Nebraska State Museum, Harold W. Manter Laboratory of Parasitology collections; HWML48446 (hapantotype); HWML48439–HWML48445 (paratypes).

*Etymology*: The specific epithet is given in honor of Dr. Richard Clopton, who, with his series of recent taxonomic papers, has established the standards of gregarine species-level systematics.

#### Remarks

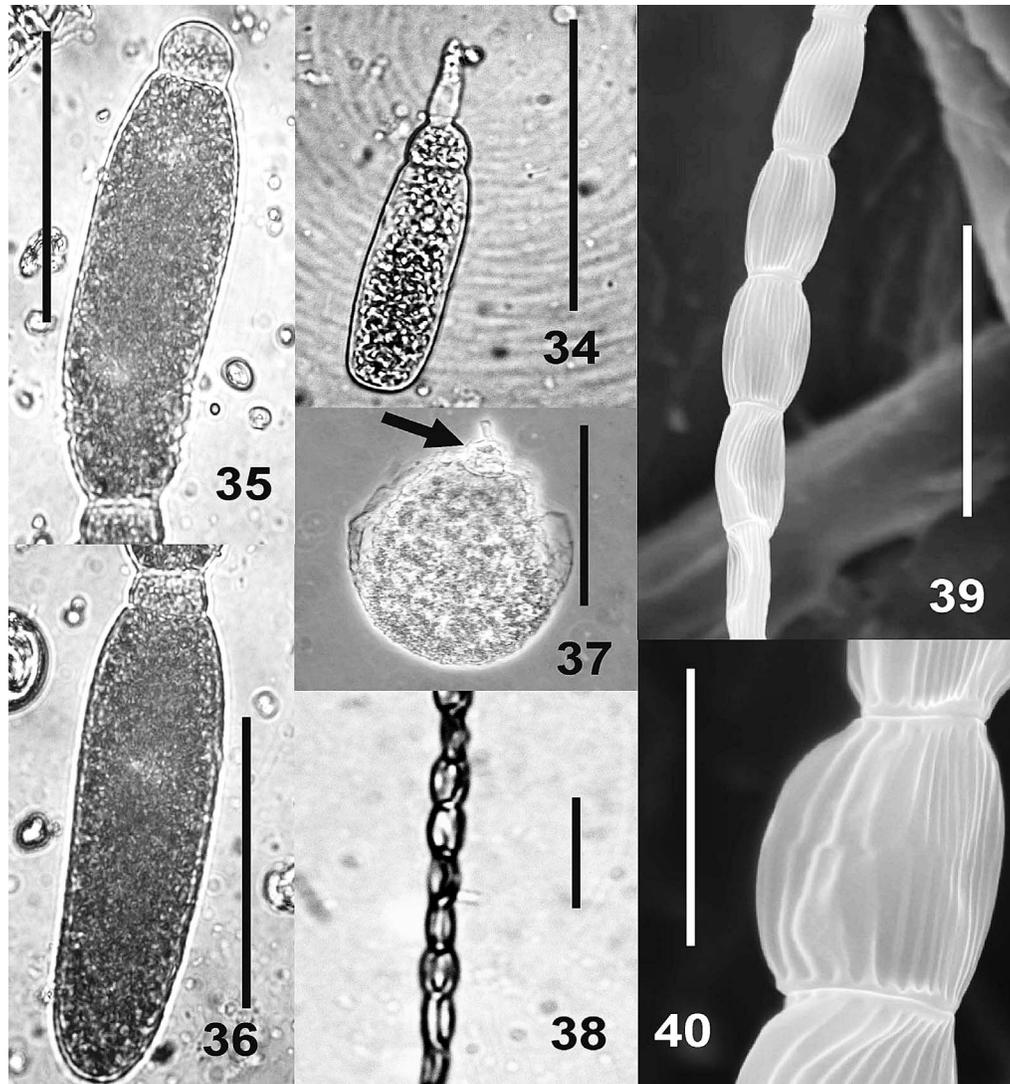
*Gregarina cloptoni* differs from other *Gregarina* spp. described from flour beetles in having a globular epimerite that often persists in the gamonts and in having larger gametocysts than *G. palori*, *G. minuta*, or *G. confusa*. In addition, among the 4 gregarine species described and redescribed from *Tribolium* species in this article, oocyst measurements differ significantly when tested by ANOVA; for lengths,  $F = 73.7$  (3, 196), widths,  $F = 50.4$  (3, 196), and thicknesses,  $F = 6.3$  (3, 196). *Gregarina cloptoni* oocysts are narrower than those of either *G. palori* or *G. minuta*, but wider than those of *G. confusa*.

#### *Gregarina confusa* n. sp.

(Figs. 34–47)

*Trophozoite* (Figs. 34, 41–43): Epimerite, a long cone. Protomerite very shallowly to broadly ovoid; deutomerite narrowly to very narrowly oblong. Measurements and correlations between measurements in Table II.

*Gamonts and association* (Figs. 35–36, 44–46): Primitive: protomerite broad to very broadly ovoid; occasionally with epimerite; deutomerite narrow to very narrowly oblong. Satellite: protomerite shallow to broadly ovoid; deutomerite narrow to very narrowly oblong. Measurements and correlations between measurements in Table II.



FIGURES 34–40. *Gregarina confusa*, n. sp. various stages. (34) Young trophozoite. Bar = 50  $\mu$ m. (35–36) Mature associated pair, primita and satellite, respectively. Bar = 100  $\mu$ m. (37) Gametocyst with sporoduct (arrow). Bar = 100  $\mu$ m. (38) Oocyst chain. Bar = 10  $\mu$ m. (39–40) SEMs of oocysts (Fig. 39, bar = 10  $\mu$ m; Fig. 40, bar = 5  $\mu$ m).

*Gametocysts* (Fig. 37): Translucent and white when shed, with a hyaline space up to 10  $\mu$ m wide surrounding inner cytoplasmic mass; subspherical to broadly ellipsoid, variable in size; 82.3 (57.0–95.0, 10.6, 15) by 75.4 (57.0–90.3, 10.9, 15).

*Oocysts* (Figs. 38–40, 47): In chains, end-to-end, with long axis parallel to chain axis but with individual cysts rotated at various angles along axis; oblong, irregularly wrinkled, indented, or compressed when dry; length 4.8 (3.3–6.3, 0.8, 50), width 2.6 (2.0–3.3, 0.3, 50), thickness 1.7 (1.0–2.6, 0.4, 50). Ultrastructurally with series of ridges parallel to long axis (Figs. 39–40).

**Taxonomic summary**

*Host*: *Tribolium confusum* Jacquelin du Val, 1868.  
*Symbiotype*: University of Nebraska State Museum, Entomology Division collection, vouchers: J. Janovy, Jr., 021–030 (10 pinned adults on points); J. Janovy, Jr., 052 (larvae in vial).  
*Host records*: *Tribolium confusum* Jacquelin du Val, 1868; larvae; laboratory infections.  
*Locality*: University of Nebraska Lincoln; School of Biological Sciences, Manter Hall, Lincoln, Nebraska, cultures.  
*Infection site*: Midgut.  
*Specimens*: University of Nebraska State Museum, Harold W. Manter

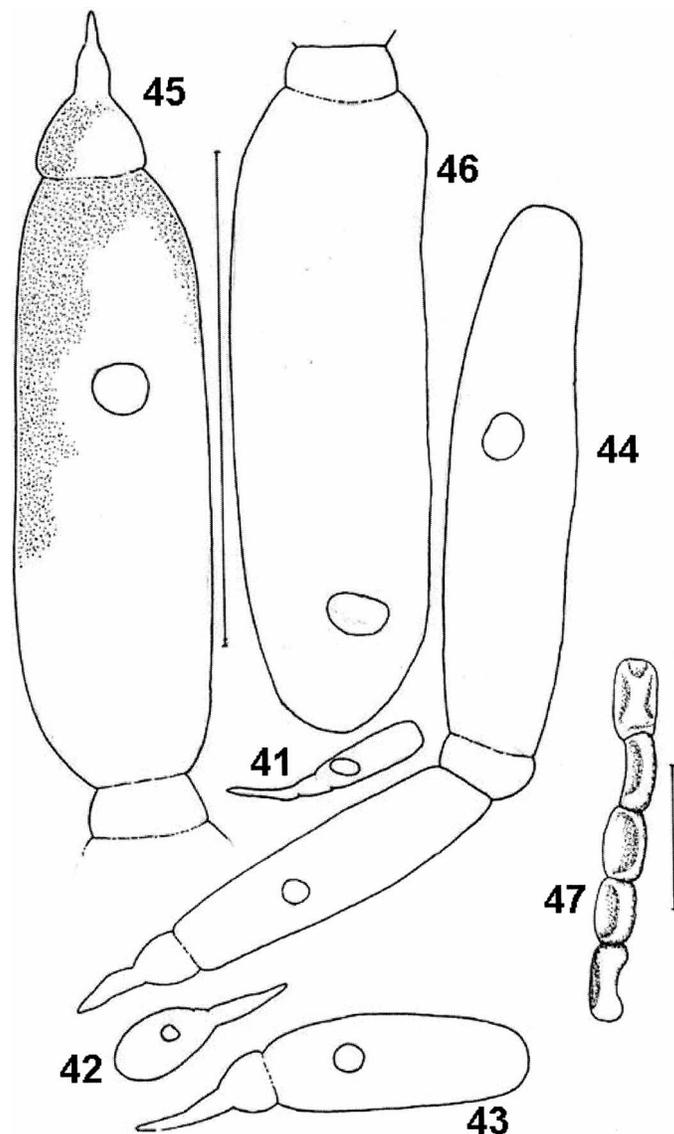
Laboratory of Parasitology collections; HWML48449 (hapantotype); HWML48447–HWML48448 and HWML48450–48451 (paratypes).  
*Etymology*: The specific epithet is derived from that of the type host.

**Remarks**

The major feature distinguishing *G. confusa* from the other described gregarines parasitizing *Tribolium* species is the elongate cone epimerite. None of the published descriptions of gregarines from flour beetles shows or indicates such an epimerite. In fresh preparations, this epimerite begins to swell into a bubble basally, likely in response to osmotic pressure, as if the membranes were relatively fragile at this particular place on the cell surface. In trophozoites, the protomerite is also relatively shorter than in the other species (TL/PL = 8.7 for *G. confusa* vs. 7.6–8.6 for the others). Finally, the *G. confusa* oocysts are smaller than those of the other species described in this article.

***Gregarina palori* n. sp.**  
 (Figs. 48–61)

*Trophozoite* (Figs. 48, 55–57): Epimerite spherical or subspherical, subequal in size to protomerite. Protomerite shallow to broadly ovoid;



FIGURES 41–47. *Gregarina confusa*, n. sp. (41–43) Trophozoites of different ages. (44) Immature associated pair. (45–46) Mature associated pair, primate and satellite, respectively. (47) Oocyst chain. Figures 41–46, bar = 100  $\mu\text{m}$ ; Figure 47, bar = 10  $\mu\text{m}$ .

deutomerite narrow to very narrowly oblong. Measurements and correlations between measurements in Table II.

**Gamonts and association** (Figs. 49–50, 58–60): Primate: epimerite sometimes present; protomerite shallow to broadly ovoid; deutomerite narrow to very narrowly oblong. Satellite: protomerite very shallow to very broadly ovoid; deutomerite oblong to very narrowly oblong. Measurements and correlations between measurements in Table II.

**Gametocysts** (Fig. 51): Subspherical, with obvious cyst wall of uniform thickness; 75.8 (52.3–104.5, 17.5, 20) by 69.1 (47.5–90.0, 16.4, 20).

**Oocysts** (Figs. 52–54, 61): In chains, end-to-end, with long axis parallel to chain axis, but with individual cysts rotated at various angles along axis; oblong, irregularly wrinkled, indented, or compressed when dry; length 6.6 (4.9–8.5, 0.8, 50); width 3.2 (2.4–4.3, 0.5, 50); thickness 1.9 (1.3–2.5, 0.3, 50). Ultrastructurally with series of ridges parallel to long axis (Figs. 53–54).

#### Taxonomic summary

**Host:** *Palorus subdepressus* Wollaston 1864.

**Symbiotype:** University of Nebraska State Museum, Entomology Di-

vision collection, vouchers: J. Janovy, Jr., 041–049 (9 pinned adults on points); J. Janovy, Jr., 054 (larvae in vial).

**Host records:** *Palorus subdepressus* Wollaston, 1864; larvae; laboratory infections.

**Locality:** University of Nebraska Lincoln; School of Biological Sciences, Manter Hall, Lincoln, Nebraska, cultures.

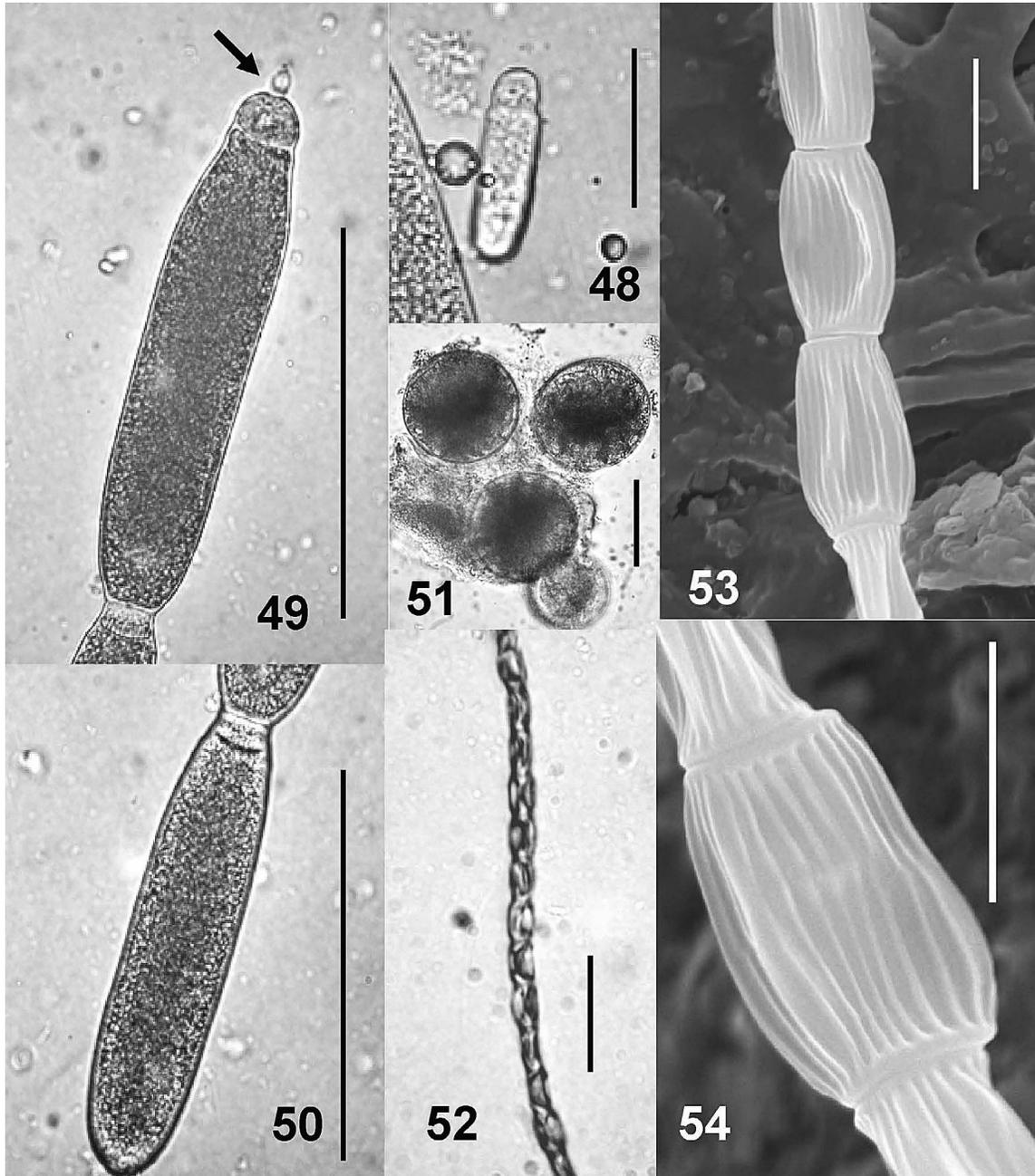
**Infection site:** Midgut.

**Specimens:** University of Nebraska State Museum, Harold W. Manter Laboratory of Parasitology collections; HWML48452 (hapantotype); HWML48453–HWML48457 (paratypes).

**Etymology:** The specific epithet is derived from that of the type host.

#### Remarks

Although the larva is designated as the stage from which this parasite is described, virtually identical parasites are found in adults in the same culture. Experimental evidence from this laboratory indicates that uninfected adults can be infected with *G. palori* oocysts from gametocysts shed by larvae (Detwiler, 2004). The gametocysts are the smaller than those of *G. confusa*, *G. cloptoni*, and *G. minuta*, but the oocysts are the largest among the 4 *Gregarina* species described in this article.



FIGURES 48–54. *Gregarina palori*, n. sp. various stages. (48) Young trophozoite. Bar = 30  $\mu\text{m}$ . (49–50) Mature associated pair, primite and satellite, respectively. Bar = 100  $\mu\text{m}$ . (51) Gametocysts. Bar = 50  $\mu\text{m}$ . (52) Oocyst chain. Bar = 10  $\mu\text{m}$ . (53–54) SEMs of oocysts showing longitudinal ridges. Bar = 5  $\mu\text{m}$  in Figures 53–54.

**EMENDED DESCRIPTION**

***Gregarina minuta* Ishii, 1914**  
(Figs. 62–74)

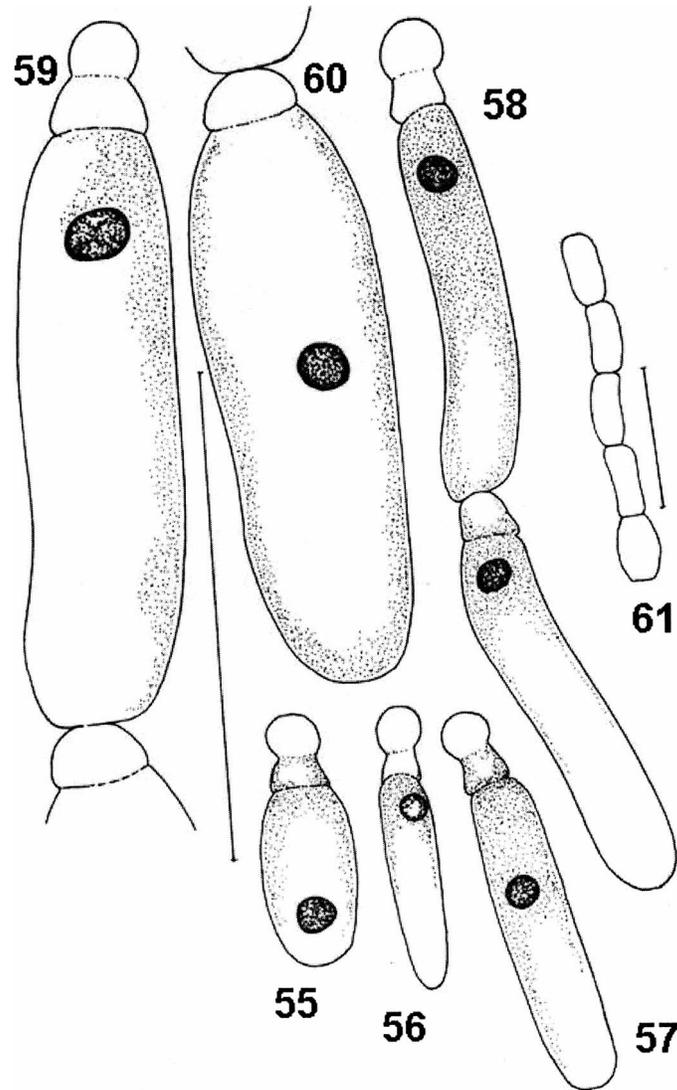
*Trophozoite* (Figs. 62, 69–70): Epimerite elongate globular or slightly conical. Protomerite ovoid to broadly ovoid; deutomerite oblong to very narrowly oblong. Measurements and correlations between measurements in Table II.

*Gamonts and association* (Figs. 63–64, 71–73): Primite: protomerite very shallow to broadly ovoid; deutomerite narrow to very narrowly

oblong. Satellite: protomerite very shallow to broadly ovoid; deutomerite narrow to very narrowly oblong. Measurements and correlations between measurements in Table II.

*Gametocysts* (Fig. 65): Translucent and white when shed, often with hyaline space up to 10  $\mu\text{m}$  wide surrounding inner cytoplasmic mass; subspherical to broadly ellipsoid, quite variable in size; 76.2 (47.5–104.6, 16, 23) by 72.5 (57.0–90.3, 11.0, 23).

*Oocysts* (Figs. 66–68, 74): In chains, end-to-end, with long axis parallel to chain axis but with individual cysts rotated at various angles along axis; oblong, irregularly wrinkled, indented, or compressed when dry; length 5.6 (4.4–6.5, 0.4, 50), width 3.4 (3.0–3.8, 0.2, 50), thickness



FIGURES 55–61. *Gregarina palori*, n. sp. (55–57) Trophozoites of different ages, showing globular epimerites. (58) Immature associated pair. (59–60) Mature associated pair, primate and satellite, respectively. (61) Oocyst chain. Figures 55–60, bar = 100 μm; Figure 61, bar = 10 μm.

1.9 (1.3–2.3, 0.3, 50). Ultrastructurally with series of ridges parallel to long axis (Figs. 67–68).

**Taxonomic summary**

*Host:* *Tribolium castaneum* (Herbst, 1797) Good, 1936 (= *Tr. ferrugineum* Wollaston, 1854).

*Symbiotype:* University of Nebraska State Museum, Entomology Division collection, vouchers: J. Janovy, Jr., 011–020 (10 pinned adults on points); J. Janovy, Jr., 051 (larvae in vial).

*Host records:* Larvae of *Tribolium castaneum* (Herbst, 1797) Good, 1936 (= *Tr. ferrugineum* Wollaston, 1854); larvae; laboratory infections.

*Locality:* University of Nebraska Lincoln; School of Biological Sciences, Manter Hall, Lincoln, Nebraska, cultures.

*Infection site:* Midgut.

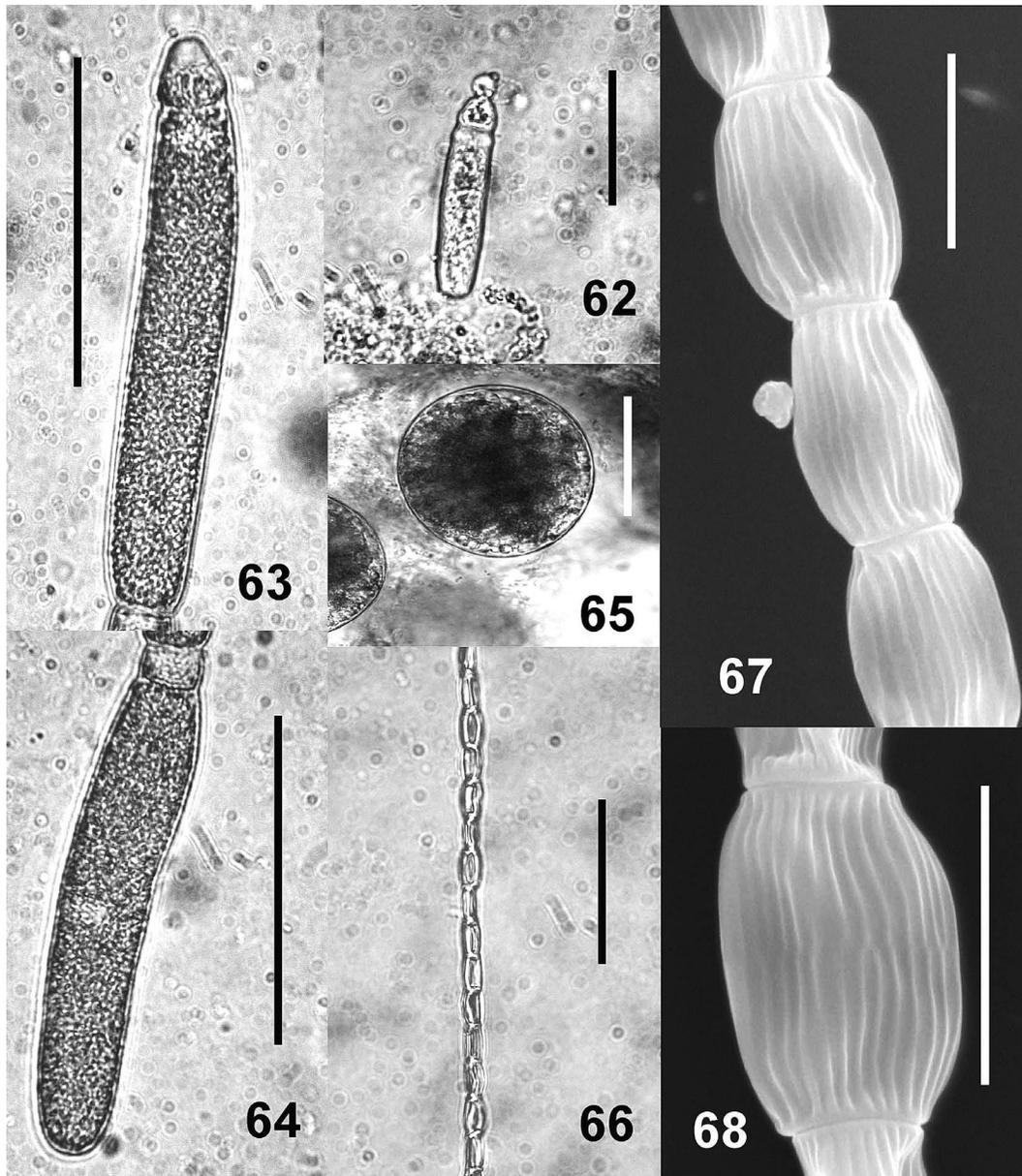
*Specimens:* University of Nebraska State Museum, Harold W. Manter Laboratory of Parasitology collections; HWML48458 (hapantotype); HWML48459–HWML48463 (paratypes).

**Remarks**

Ishii (1914) described *G. minuta* from the adults of *Tr. ferrugineum*, a host species now considered synonymous with *Tr. castaneum*. This

description actually refers to Figure 2b of Ishii (1914); Figure 2a in the Ishii (1914) article is a different species, *G. triboliorum*, and Figure 2c from that article could be a singlet of either species, although it is most likely one of *G. minuta*. (See Watwood et al. [1997] for resolution of the confusion surrounding these 2 species in the same host beetle.) A complete discussion of host nomenclatural history is found in Good (1936), and the decision to designate larval *Tr. castaneum* as the type host is based on that discussion. Experimental evidence from this laboratory indicates that uninfected adult beetles can be infected with *G. minuta* oocysts from gametocysts shed by larvae (Detwiler, 2004). The present description adds the Clopton (1999, 2004a) extended morphometric data set and shape nomenclature; epimerite shape, gametocyst and oocyst description, and measurements; and SEM micrographs of oocysts to the description. With these measurements and descriptors, *Gregarina minuta* is thus distinguished clearly from *G. triboliorum* Watwood et al., 1997, another species found in *Tr. castaneum*. *Gregarina triboliorum* not only loses the satellite protomerite very early in development but also exhibits a finely granular cytoplasm that is easily recognized as different from the coarser cytoplasm of *G. minuta* on stained smears. The elongate cone-shaped epimerite of *G. minuta* is fragile near the base, and even in *Tenebrio* saline, its membrane tends to form a bubble, evidently from uptake of water.

Measurements of the emended description are larger than those re-



FIGURES 62–68. *Gregarina minuta*, various stages. (62) Young trophozoite. Bar = 30  $\mu\text{m}$ . (63–64) Associated pair, primate and satellite, respectively. Bar = 100  $\mu\text{m}$ . (65) Gametocyst. Bar = 50  $\mu\text{m}$ . (66) Oocyst chain. Bar = 25  $\mu\text{m}$ . (67–68) SEMs of oocysts showing longitudinal ridges. Bar = 5  $\mu\text{m}$  in Figures 67–68.

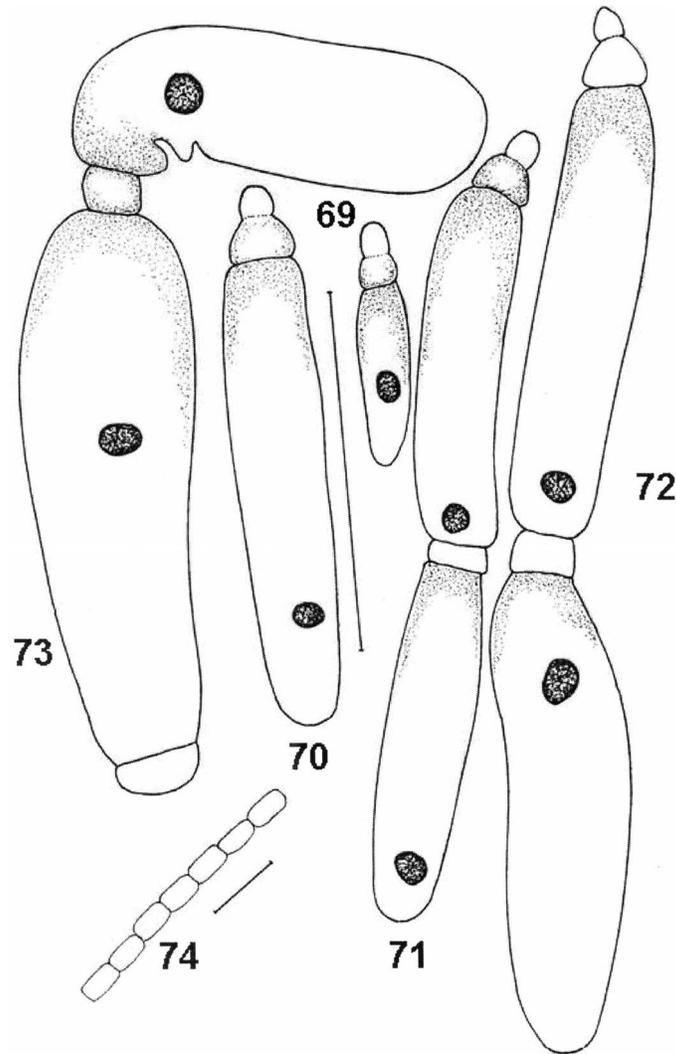
ported by Ishii (1914) for *G. minuta*, but Ishii (1914) does not indicate whether adult or larval hosts were used in the study, and neither numbers of observations and ranges nor ratios suggesting body proportions are given. The decision to amend the Ishii (1914) description, rather than describe another new species from this same host species, is based on the persistent satellite protomerite in the present study material and the uncertainty of the number of measurements and their ranges in the original description.

A number of other gregarine taxa have been described from *Tr. castaneum*, especially by workers in India (e.g., Ghose et al., 1986; Gupta and Haldar, 1987; Ghose and Haldar, 1989; Sengupta et al., 1991; Saha et al., 1995). In general, it is possible to distinguish these genera and species from ones reported in this article, but in some cases the distinctions are not clear. Gregarine descriptions published before Clopton (1999, 2004a) do not include all of the measurements and characters now commonly used and often do not distinguish between primites and

satellites. In the case of *G. minuta*, oocyst shape separates this species from all of those described by the Indian workers cited above.

### DISCUSSION

The major contribution of this article is the provision of a described and domesticated system for the study of several species of related parasites among a set of congeneric and confamilial host species, all of which are easily maintained in culture. Thus, we are in a position to begin asking whether the 2 host and parasite phylogenies are congruent, or whether there is evidence for independent acquisition of parasite taxa by host taxa. This question is not a particularly new one, nor is it unique to coleopterans and their apicomplexan parasites. With this taxo-



FIGURES 69–74. *Gregarina minuta*. (69–70) Trophozoites of differing ages. (71–72) Associated gamonts, showing persistent epimerites. (73) Mature associated gamonts. (74) Oocyst chain. Figures 69–73, bar = 100  $\mu\text{m}$ ; Figure 74, bar = 10  $\mu\text{m}$ .

onomic work, however, one can begin to address the question of whether phylogenies constructed after the fact of their origin can actually hide attributes that either influenced that origin or could constrain a host species list for a particular parasite species, thus potentially directing future evolutionary change. For example, with the gregarine-flour beetle system, we can determine experimentally whether a parasite from 1 host species can, if given the opportunity, infect related hosts. Multispecies systems amenable to such experimental exploration of avenues for evolutionary change are not common, especially when one considers logistical constraints associated with the potential use of many reasonably well-studied taxa as laboratory models (e.g., see Snyder and Tkach, 2001; León-Règagnon and Brooks, 2003; Caira et al., 2005).

Anyone familiar with gregarine systematic literature will immediately recognize that the descriptions herein are based at least partly on the principle of “new host studied, new parasite discovered.” This principle has guided gregarine taxonomy for many decades and, although it is not necessarily an advisable one to follow in all instances (e.g., see Watwood et al., 1997),

it is an acceptable one in the present case. We contend that with respect to gregarines in flour beetles, it is more important at this stage in the development of our understanding of gregarine biology to have a scientific name, a describer and date, and a set of characters consistent with current practice, all in print, than it is to engage in years of research, trying to establish whether species are valid. Oocyst lengths, widths, and thicknesses differ significantly among the described species, and there are certainly epimerite shape differences between some of the species. With these descriptions, it is now possible to do the experimental infection work that could reveal host-induced variation; avenues for, and constraints on, colonization; effects of host-induced variation in parasite attributes; and possible pathogenic effects of parasites in unusual hosts. Most important, however, this work now allows us to determine whether, within a group of related parasites, generalists (infecting 2 or more host species) and specialists (restricted to a single host species) occur. Much of this kind of research, however, must await appropriate molecular studies on this group of parasites.

In addition to the above issues regarding gregarine system-

atics, it is also necessary to recognize that gregarine morphology is not easy to describe satisfactorily, and indeed, the conventions of measurement may lead a naïve reader astray. The growing body of recent taxonomic work by Clopton and co-workers (Clopton, 1999, 2000, 2004a, 2004b; Clopton et al., 2004) has done much to resolve this problem, but there is really no way to avoid certain descriptive difficulties associated with gregarine development. For example, the terms “trophont” or “trophozoite” seem to be well-defined ones, but this life-cycle stage is so designated because the individual cells are neither obviously associated nor otherwise exhibit visual evidence of commitment to gametogenesis. Association, again by definition, signifies that the cells have become “gamonts” or “gametocytes.” But some species are precocious, associating early in development, whereas others associate late. Phasing of association has been mentioned as a taxonomic character at both the generic and family levels (Kula and Clopton, 1999; Clopton, 2000). The present study suggests that the timing of association is variable enough within a group of congeners so that it could also be used at even the species level, provided structural features map onto developmental ones. However, phasing of association could also be influenced by host species and diet, a facet of gregarine biology that has yet to be explored but could easily be investigated with the flour beetle–gregarine system.

The oocyst SEMs in this article are also an important contribution to the gregarine literature. From these pictures, we discover that oocysts seeming to differ primarily in their outlines as viewed from one of the broadest dimensions, especially under the light microscope suspended in glycerin, can in fact be quite distinct in their surface architecture. The examination of oocyst measurements and apparent structure under different observational environments also reveals information of use in systematics. Insofar as can be determined using light microscopy, oocysts of the *Gregarina* species studied are irregularly wrinkled, in addition to being ridged, when observed dry, as they would occur in nature. When flooded with glycerin, however (see Clopton et al., 1991), these same oocysts appear dolioform and relatively smooth. Previous studies also suggest that gregarine oocysts are also notoriously uniform in size and shape, thus in principle, are good taxonomic characters (Clopton et al., 1991). The present work suggests that measurement conditions are of prime importance in assessing the differences between taxa in this regard.

Finally, although all the parasite species described and re-described in this article are from the larval hosts, in culture, all of the adults are also infected with gregarine parasites that are generally indistinguishable from those in the larvae. Detwiler (2004) showed experimentally that gregarines from all the studied flour beetle species' larvae will infect their respective species' adults but was not able to do the reciprocal crosses because of logistical constraints. This work will be presented in detail in the near future, but, for the present, parasites described in the current article should probably be considered ones of both adults and larvae of their respective host species. In the case of holometabolic hosts, this question of cross-stadial infectivity is an important one because of the Clopton et al. (1992) demonstration of host stadium specificity in gregarine parasites of the confamilial *Tenebrio molitor* in contrast to the Watwood et al. (1997) experimental demonstration of both cross-stadial and cross-specific infectivity in *Gregarina tribo-*

*liorum* from *Tr. confusum* and *Tr. castaneum*. *Tribolium madens*, also obtained from the USDA Grain Marketing and Production Center in Manhattan, Kansas, at the same time as the other species used in the present study, has never been infected with any gregarines, either in the adult or larval stage, in this laboratory. Attempts to infect it with gregarines from other *Tribolium* species have not been successful, but those studies are still in a very preliminary stage. The varying degrees of host specificity among gregarines within this group of tenebrionid beetles thus adds to the value of the system as material to study the mechanisms by which host and parasite lineages develop over time.

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