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REVISION OF THE GENUS *TOROGREGARINA* AND DESCRIPTION OF *TOROGREGARINA SPHINX* N. SP. (APICOMPLEXA: EUGREGARINIDA) FROM A MISSOURI RIVER BANK BEETLE, *BEMBIDION LAEVIGATUM* (COLEOPTERA: CARABIDAE), IN SOUTHEASTERN NEBRASKA

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ABSTRACT: *Torogregarina* is revised to clarify the nature of the epimerite–protomerite complex and the method of gametocyst dehiscence, removed from the family Gregarinidae, and placed among the Hirmocystidae. *Torogregarina sphinx* n. sp. (Apicomplexa: Eugregarinida) is described from *Bembidion laevigatum* (Coleoptera: Carabidae) collected along the Missouri River near Peru, Nemaha County, Nebraska. Measurements are means in micrometers. Association is precocious and caudofrontal. Primite epimerite depressed ovoid and persistent through syzygy; length 25.2, width 41.0. Protomerite a high toroid collar between epimerite and deutomerite; length 13.5, width 35.4. Deutomerite obovoid; length 137.3, width 90.7. Total length including epimerite 172.2. Satellite epimerite–protomerite complex concentrically formed by a depressed ovoid epimerite within a transversely oblong protomerite, the protruding dome of the epimerite fitting into a posterior depression on the primite and the transverse anterior margin of the protomerite mating with the posterior margin of the primite to form the syzygial junction; length 25.0, width 48.8. Deutomerite obovoid; length 113.5, width 63.5. Total length 138.6. Gametocysts roughly spherical; diameter 142.9. Gametocysts mature and dehisce by simple rupture, extruding oocysts in a single mass without an epispore packet. Oocysts axially symmetric, dolioform in dorsal aspect, concavoconcave in transverse plane, uniform in size and shape; length 5.2, terminal width 2.4, equatorial width 3.1.

The gregarines are a speciose and ubiquitous group of intestinal parasites reported from nearly all invertebrate groups. There are approximately 1,600 named species of gregarines, but Levine (1988) estimated that over 850,000 species remain to be discovered. Biotic survey remains the primary focus of gregarine study and the potential diversity of the group is reflected by the rate of species discovery and description. Over 89% of known gregarine species have been described in this century; approximately half of all named gregarine species have been described since 1950 (Levine, 1988).

In theory, monotypic genera are hypotheses of generic relationship and potential synapomorphy that are tested by the discovery of additional species. The Eugregarinorida Léger, 1900 is rife with such generic hypotheses. The superfamily Gregarinicae Chakravarty, 1960 comprises 7 families and 46 genera of which 22 are monotypic (Gregarinidae Labbé, 1899, 15 described genera, 8 monotypic; Metameridae Levine, 1979, 4 described genera, 3 monotypic; Didymophyidae Léger, 1892, 1 described genera, polytypic; Cephalolobidae Théodoridès and Desportes, 1975, 2 described genera, 1 monotypic; Uradiophoridae Grassé, 1953, 5 described genera, 2 monotypic; Cephaloidophoridae Kamm, 1922, 3 described genera, all polytypic, and; Hirmocystidae Grassé, 1953, 16 described genera, 8 monotypic).

Gregarinidae and Hirmocystidae are particularly unstable because fully half of the component genera are monotypic. Biotic survey and subsequent taxonomic studies of the group simultaneously test our current hypotheses of relationship within Gregarinidae and Hirmocystidae and among their component genera, as well as refine our understanding of gregarine α-taxonomy. In the end, the derivation of relationship among the families of Gregarinicae or any other higher level systematic hypothesis within the gregarines depends upon rigorous testing and clarification of monotypic genera through biotic survey and α-taxonomic study.

During an on-going biotic survey of the gregarine parasites of North American insects, a heretofore unknown gregarine species was discovered in populations of *Bembidion laevigatum* Say, 1823 (Coleoptera: Carabidae) inhabiting the banks of the Missouri River in southeastern Nebraska. The gregarine populations recovered from *B. laevigatum* are taxonomically distinct from known gregarine species and represent a second species of *Torogregarina* Geus, 1969, a monotypic genus originally described from Germany. The genus *Torogregarina* is confirmed, revised to clarify the generic diagnosis, removed from the family Gregarinidae, and placed among the Hirmocystidae, and a new species of *Torogregarina* is proposed herein.

MATERIALS AND METHODS

Bembidion laevigatum adults were collected from the bank of the Missouri River near Peru, Nemaha County, Nebraska during March–May 1996. Locality coordinates were determined using an Eagle Explorer Global Positioning Satellite locator. Beetles were transported to the laboratory and maintained in 4-L glass jars with a fine earth substrate. Fish food (TetraMin) and water-soaked dental rolls were provided daily to supply food and water, respectively. All beetles were either preserved as permanent specimens or examined for gregarine infection within 48 hr of collection. *Bembidion laevigatum* adults were eviscerated and their alimentary canals dissected in insect muscle saline (Belton and Grundfest, 1962). Smear preparations of host intestinal tissue and lumenal contents were either fixed in AFA (ethanol, formalin and acetic acid [Galigher and Kozloff, 1971]), rinsed in 70% ethanol, and stained in Semichon's acetocarmine (Semichon, 1924) (2 min each reagent), or simultaneously fixed and stained for 2 min in Semichon's acetocarmine (Semichon, 1924). Fixed and stained smears were dehydrated in ethanol, cleared in xylene, and mounted in Damar balsam (Galigher and Kozloff, 1971). Gametocysts were collected from dissected recta and transferred into individual wells of a Miniwell® assay plate (Nunclon Miniwell® minitray plate; 60 conical, flat-bottomed, 10-μl wells; Nunclon 439225, Nunc, Inc., Denmark). Water was added to the margins of the culture plate to provide humidity, and the gametocysts were held for maturation and dehiscence. Oocyst structure and dimensions were taken from fresh preparations of oocysts suspended in water or glycerin. Oocysts rotated freely in glycerin preparations and the full 3-dimensional structure was observed.

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Widths of protomerites and deutomerites were taken at the widest points. Measurements were taken on fixed specimens and are presented in μm as range values followed by means, standard deviations, and sample sizes in parentheses. Terminology for parasite ontogenetic stages follows that proposed by Levine (1971). Terminology for shapes of planes and solids is consistent with that suggested by the Systematics Association Committee for Descriptive Biological Terminology (Anonymous, 1962).

Observations and measurements were made using an Olympus EH-2 and BioScan Optimas version 4.1 image analysis software (BioScan Inc., Edmonds, Washington) using input from a Javelin Ultrachip high-resolution camera. Drawings were made using digitized images of live gregarines supplemented with the aid of a camera lucida. Photographs were taken with an AGFA ActionCam digital camera through an Olympus B-Max 50 compound microscope with 20× and 40× universal plan-apochromatic objectives and differential interference contrast prisms.

DESCRIPTION

Torogregarina Geus, 1969
(Fig. 1)

Revised diagnosis: Eugregarinida Léger, 1892 sensu strictu Levine et al. (1980); Septatina Lankester, 1885, sensu strictu Levine et al. (1980); Gregarinicae Chakravarty (1960); Hirmocystidae Grassé, 1953, with the characters of the genus *Torogregarina* Geus, 1969: epimerite persistent in associated gamonts, epimerite of gamont large, ovate; protomerite a broad, basal swelling or toroid tumidus; association precocious, caudofrontal; gametocysts round, dehiscing by simple rupture; oocysts dolioform, not released in chains, without enclosing epispore packet.

Taxonomic summary

Type species: Torogregarina stammeri Rauchalles in Geus, 1969.

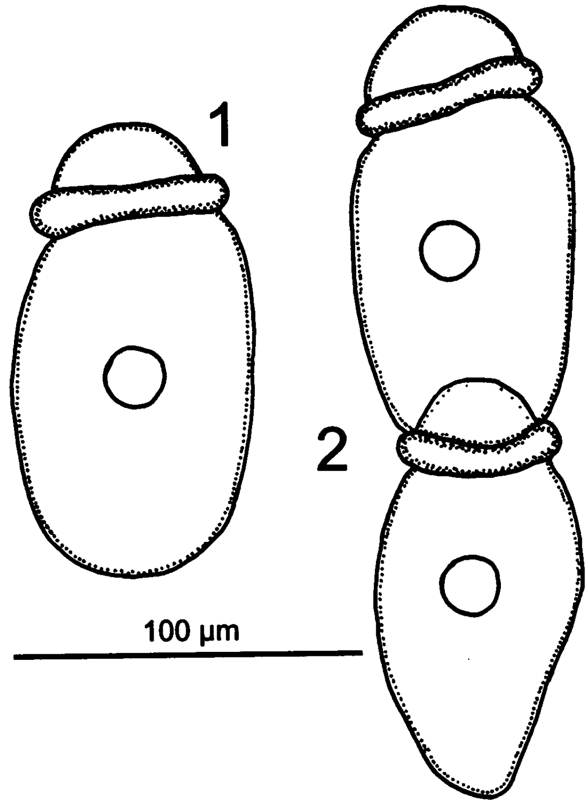
Remarks

Geus (1969) published the species *T. stammeri* to typify a monotypic genus of septate gregarines characterized by a toroid swelling or collar just anterior to the protomerite–deutomerite septum (Figs. 1, 2). The original diagnosis of *Torogregarina* was based on limited numbers of trophozoites (n = 8), gamonts (n = 10), and a single gametocyst collected from 4 specimens of *Nosodendron fasciclare* (Olivier, 1790) (Coleoptera: Dermestoidea: Nosodendridae) (Geus, 1969). The discovery of a second species of *Torogregarina* confirms the generic hypothesis of Geus (1969). Observations of gregarines and gametocysts collected from 108 host beetles in the current study suggest a reinterpretation and clarification of the genus *Torogregarina* and its systematic placement within the Gregarinicae.

Geus (1969) interpreted the toroid swelling or collar as a basal extension of the protomerite. He made no mention of an epimerite. Trophozoites have been observed at all stages of development in the current study and there is no evidence of a transitory epimerite. However, the "broad, basal swelling or toroid tumidus" is distinguished by the indistinct anterior septum and distinct posterior septum and is anatomically consistent with a protomerite. Thus observations of the species described here indicate that the broad, hemispherical protomerite described by Geus (1969) is actually a persistent epimerite and the "broad, basal swelling or toroid tumidus" is a modified protomerite.

Geus (1969) provided descriptions of gametocysts and oocysts and although he did not observe oocyst dehiscence, he surmised that oocysts were released from the gametocyst by simple rupture of the gametocyst wall. The current study confirms this supposition: the oocysts of *Torogregarina* are released from the gametocyst in clumps through simple rupture of the gametocyst wall.

Based on these observations, Geus's (1969) original diagnosis of *Torogregarina*, "protomerite of gamont with a broad, basal swelling or toroid tumidus; association precocious, caudofrontal; gametocysts round, dehiscing by simple rupture; oocysts dolioform" is emended to reflect interpretations based on new evidence. *Torogregarina* Geus, 1969: epimerite persistent in associated gamonts, epimerite of gamont large, ovate; protomerite a broad, basal swelling or toroid tumidus; gametocysts round, dehiscing by simple rupture; oocysts dolioform, not released in chains, without enclosing epispore packet.



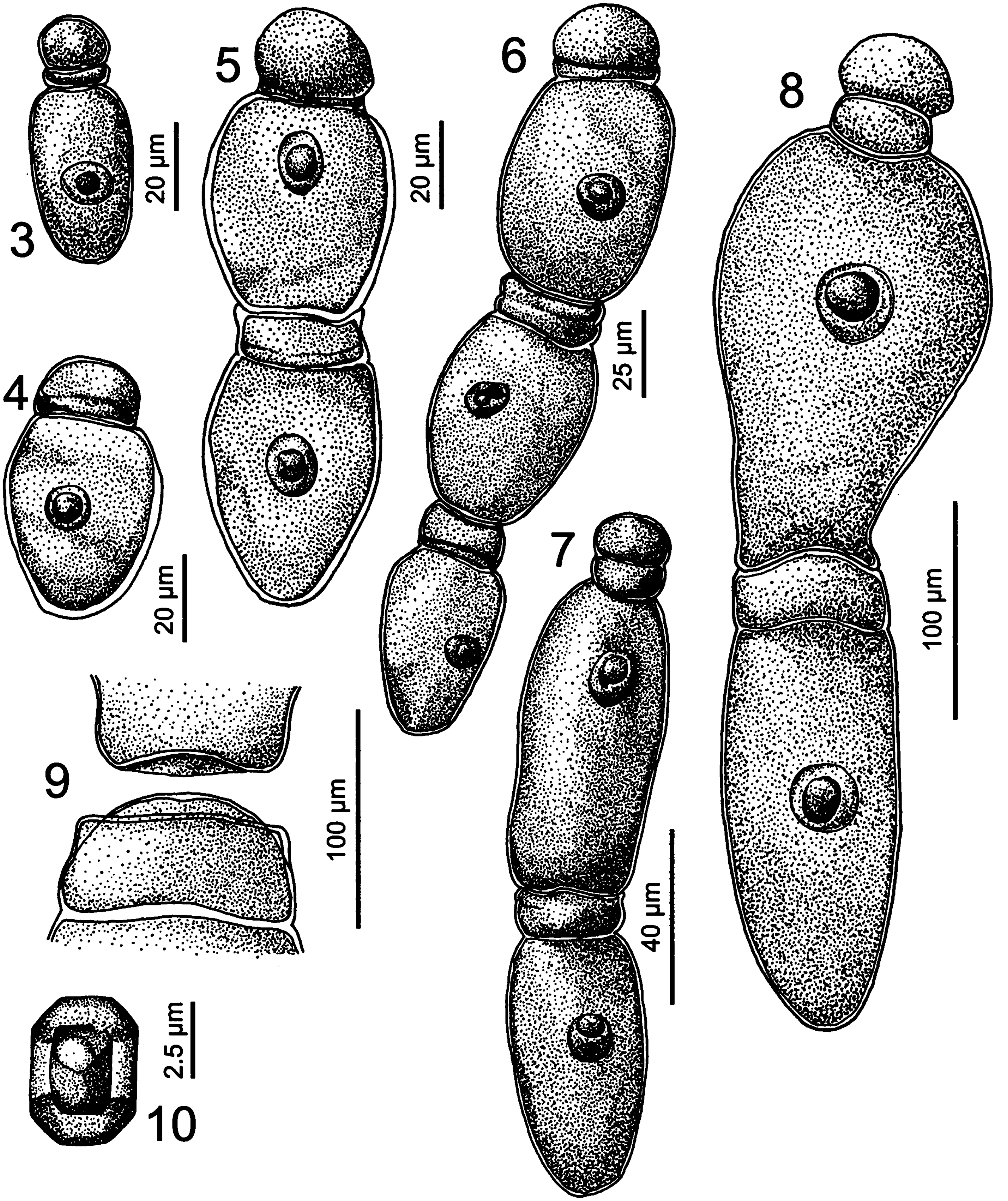
FIGURES 1, 2. *Torogregarina stammeri.* (Redrawn from Geus, 1969.) **1.** Trophozoite. **2.** Association.

The genus *Torogregarina* was originally placed within the Gregarinidae by Geus (1969) and this placement was retained by Levine (1988). The family Gregarinidae is distinguished from the allied family Hirmocystidae by differences in oocyst dehiscence. The Gregarinidae dehisce through sporoducts while the Hirmocystidae dehisce through simple rupture of the gametocyst wall. Observations of the species described here confirm Geus's (1969) supposition that the oocysts of *Torogregarina* dehisce through simple rupture of the gametocyst wall, clearly indicating their correct placement within Hirmocystidae rather than Gregarinidae.

Torogregarina sphinx n. sp.
(Figs. 3–10)

Description

Trophozoite (Figs. 3–6): Developing solitary trophozoites rare (Figs. 3, 4), attached to host ventricular epithelium, association precocious (Figs. 5, 6). Epimerite widely depressed ovoid to depressed ovoid or basidioform, posterior margin recurving to junction with protomerite, without a visible septum, but clearly differentiated by accumulated cytoplasmic granules; length (LE) 8.3–42.2 (19.5, ±9.2, 47), width (WE) 20.1–61.7 (35.1, ±11.4, 47), WE:LE 1.25–3.33 (1.96, ±0.58, 47). Protomerite narrowly transverse elliptoid to toroid, forming a depressed collar between protomerite and deutomerite; length (LP) 5.0–22.7 (10.9, ±4.7, 47), width (WP) 18.8–57.5 (33.3, ±10.9, 47), WP:PL 1.50–6.24 (3.29, ±1.04, 47). Protomerite–deutomerite septum clearly marked and constricted. Deutomerite obovoid, anterior margin with a shallow depression or concave sinus at junction with protomerite; length (LD) 44.6–146.8 (86.6, ±29.9, 47), width (WD) 29.1–92.8 (57.2, ±19.0, 47), WD:LD 0.42–1.00 (0.68, ±0.17, 47). Total length including epimerite (TL) 24.6–188.1 (111.7, ±41.6, 47). Indices: LP:TL 0.05–0.56 (0.12, ±0.10, 47); LD:TL 0.58–4.19 (0.89, ±0.71, 47); LP:LD 0.07–0.24 (0.13, ±0.05, 47); WP:WD 0.42–0.74 (0.59, ±0.07, 47). Nucleus widely ellipsoid, typically medial and equatorial; length (LN) 11.2–31.0 (20.4,



FIGURES 3–10. *Torogregarina sphinx,* n. sp. **3.** Young, solitary trophozoite. **4.** Young, solitary trophozoite. **5.** Trophozoites in precocious association. **6.** Trophozoites in precocious multiple association. **7.** Early gamonts in association. **8.** Gamonts in association. **9.** Detail of primite–satellite syzygial junction. **10.** Oocyst.

±5.3, 47), width (WN) 12.8–29.6 (18.4, ±4.9, 47), WN:LN 0.70–1.52 (0.92, ±0.16, 47); with a single eccentric karyosome; length (LK) 5.4–25.2 (14.4, ±5.0, 47), width (WK) 6.1–22.2 (12.4, ±4.2, 47), WK:LK 0.37–1.31 (0.89, ±0.20, 47). Trophozoites typically form precocious and often multiple associations, with protomerite–deutomerite septa of both primites and satellites clearly marked (Figs. 5, 6).

Association (Figs. 5–9): Precocious and leading to a period of continued growth; caudofrontal, syzygial pairs and gametocysts located between host ventricular peritrophic membrane and posterior ventricular epithelium. Epimerite–protomerite complex persistent in gamonts but clearly dimorphic among primites and satellites (Figs. 7, 8).

Primite (Figs. 7, 8): Epimerite widely depressed ovate to depressed ovate, persistent through maturity and syzygy; LE 14.0–38.3 (25.2, ±6.5, 40), WE 22.3–55.1 (41.0, ±8.0, 40), WE:LE 1.17–2.48 (1.69, ±0.34, 40). Protomerite transversely depressed ovate to toroid, forming a high collar between epimerite and deutomerite, often narrower than epimerite in mature individuals, producing a basidioform protomerite–epimerite complex; LP 7.3–23.0 (13.5, ±4.0, 40), WP 20.1–46.0 (35.4, ±7.0, 40), WP:PL 1.60–5.97 (2.77, ±0.82, 40), width at protomerite–deutomerite septum (WS) 18.2–56.6 (35.2, ±8.6, 40); with strong anterior and posterior constrictions at protomerite–epimerite junction and protomerite–deutomerite septum. Deutomerite obovoid, anterior margin with a shallow depression or concave sinus at junction with protomerite, with a slight posterior depression at syzygial junction with satellite; LD 63.0–236.0 (137.3, ±39.4, 40), WD 24.0–151.5 (90.7, ±29.5, 40), WD:LD 0.34–0.97 (0.67, ±0.15, 40). Total length including epimerite 90.2–298.0 (172.2, ±43.2, 40). Indices: LP:TL 0.05–0.12 (0.08, ±0.02, 40), LD:TL 0.70–0.97 (0.79, ±0.06, 40), LP:LD 0.06–0.17 (0.10, ±0.03, 40), WP:WD 0.26–0.84 (0.42, ±0.12, 40). Nucleus widely elliptic; LN 14.9–35.9 (25.3, ±4.8, 40), WN 9.8–34.4 (22.9, ±6.3, 40), WN:LN 0.45–1.46 (0.90, ±0.17, 40); placement typically medial in the anterior third of the deutomerite; with a single eccentric karyosome; LK 10.3–26.0 (16.8, ±4.4, 40), WK 7.3–28.3 (15.2, ±5.7, 40), WK:LK 0.56–1.36 (0.90, ±0.20, 40).

Satellite (Figs. 7–9): Epimerite–protomerite complex concentrically formed by a depressed ovoid epimerite enveloped by an expanded transversely oblong to transversely elliptic protomerite, the protruding dome of the epimerite fitting into a slight posterior depression on the primite and the transverse anterior margin of the protomerite mating with the posterior margin of the primite to form a syzygial junction (Fig. 9); LP 7.7–42.0 (25.0, ±8.1, 40), WP 19.4–80.1 (48.8, ±14.1, 40), WP:LP 1.41–3.39 (2.03, ±0.45, 40); with strong anterior and posterior constrictions at syzygial junction and protomerite–deutomerite septum; width of syzygial junction (WSyz) 16.6–69.4 (41.6, ±12.2, 40), WS 15.9–77.8 (45.0, ±14.7, 40). Deutomerite obovoid, anterior margin with a shallow bulge or convex sinus at junction with protomerite; LD 39.0–195.4 (113.5, ±38.3, 40), WD 17.4–105.3 (63.5, ±20.0, 40), WD:LD 0.36–0.81 (0.57, ±0.10, 40). Total length 46.3–236.9 (138.6, ±45.0, 40). Indices: LP:TL 0.12–0.24 (0.18, ±0.03, 40), LD:TL 0.75–0.87 (0.82, ±0.03, 40), LP:LD 0.14–0.32 (0.23, ±0.05, 40), WP:WD 0.55–1.11 (0.79, ±0.12, 40). Nucleus roughly spherical; LN 8.6–32.3 (21.0, ±5.5, 40), WN 7.1–29.7 (19.6, ±5.7, 40), WN:LN 0.46–1.56 (0.95, ±0.19, 40); placement typically medial in the anterior third of the deutomerite; with a single eccentric karyosome; LK 5.9–24.2 (14.1, ±4.7, 40), WK 5.8–25.1 (13.4, ±4.9, 40), WK:LK 0.65–1.55 (0.96, ±0.18, 40).

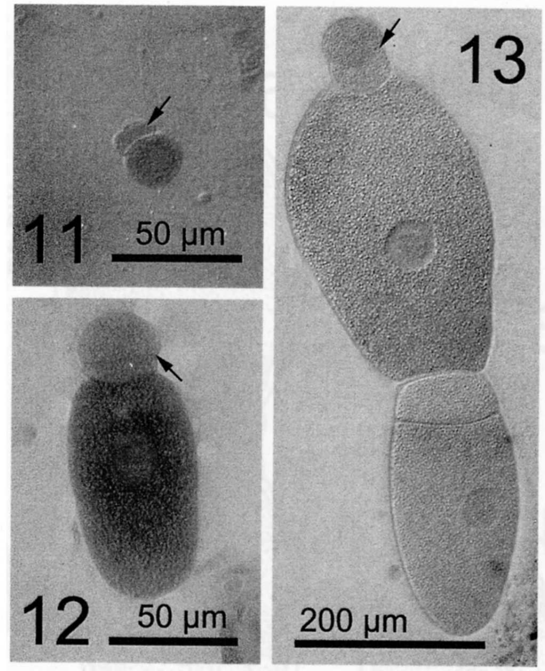
Gametocysts: White to amber, roughly spherical; diameter 123.3–162.7 (142.9, ±13.3, 33); hyaline coat absent. Gametocysts mature within 72–96 hr and dehisce by simple rupture. Oocysts are extruded in a single mass rather than in chains, no epispore packet was observed.

Oocysts (Fig. 10): Axially symmetric, dolioform in dorsal aspect, concavoconcave in transverse plane, very uniform in size and shape; length 4.5–6.1 (5.2, ±0.4, 50), terminal width 1.9–2.8 (2.4, ±0.2, 50); equatorial width 2.4–3.7 (3.1, ±0.3, 50).

Taxonomic summary

Host: Bembidion laevigatum Say, 1823 (Insecta: Coleoptera: Carabidae: Bembidiini).

Symbiotype: One symbiotype specimen is deposited Division of Entomology, University of Nebraska State Museum, Lincoln, Nebraska. The symbiotype is identified with a collection label "U.S.A. NE. Nemaha Co. Peru, Missouri River Bank 10-V-1996 Coll. R. E. Clopton," and a blue voucher label "RESEARCH PROJECT Voucher Specimen."



FIGURES 11–13. *Torogregarina sphinx*, n. sp. as revealed by differential interference microscopy. Arrows indicate epimerite–protomerite junctions. **11.** Young, solitary trophozoite. **12.** Young, solitary trophozoite. **13.** Mature gamonts in association.

An additional 30 voucher specimens are retained in the author's personal collection.

Host records: Bembidion laevigatum; adults.

Locality: N40°29′04.5″, W95°41′53.7°, Missouri River at Peru, Nemaha County, Nebraska.

Infection site: Trophozoites and gamonts were observed along the length of the ventriculus, anterior to the ileum and the attachment of the Malpighian tubules. All endogenous life-cycle stages were observed between host ventricular peritrophic membrane and ventricular epithelium. Gametocysts were collected from the ileum and rectum.

Prevalence: Eighty-four percent (91 of 108 beetles examined postmortem).

Specimens deposited: The holotype slide is deposited in the Harold W. Manter Laboratory for Parasitology (HWML), Division of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska. The holotype is an association on slide HWML 93253 (author's slide REC960010) and is marked by an etched circle. The remaining trophozoites, gamonts, and associations on HWML 93253 are paratypes. Trophozoites, gamonts, and associations on slides REC960006–REC960009 and REC960011–REC960113 are paratypes retained in the author's personal collection.

Etymology: The curious nature of the epimerite–protomerite complex in *Torogregarina* has not been well understood; thus, the generic placement of this parasite remained enigmatic for almost a full year. The specific epithet associates this parasite with the mythical riddling monster of Thebes and commemorates a year-long taxonomic puzzle.

Remarks

Torogregarina sphinx is the second species described in the genus and is distinguished from the type species, *T. stammeri*, by differences in gamont and oocyst morphology. Gamonts of *T. sphinx* are generally larger than those of *T. stammeri*, although the protomerites of *T. sphinx* are distinctly smaller than those of *T. stammeri* and differ dramatically in their morphometric relationship to other anatomical structures (Table I). These species are most readily distinguished by differences in their epimerite–protomerite complexes (compare Figs. 1, 2 with Figs. 3–13). The epimerite–protomerite complex is isomorphic in gamonts of *T. stammeri* and generally takes the form of a hemispherical epimerite with a short, basal, toroid protomerite that is wider than the epimerite (Fig.

1). In contrast, the epimerite–protomerite complex is dimorphic in gamonts of *T. sphinx*. Mature primites of *T. sphinx* possess a characteristically basidioform epimerite–protomerite complex formed by a widely obovoid epimerite with a transversely oblong, basal, toroid protomerite that is narrower than the epimerite (Figs. 5–8, 13). Mature satellites possess an epimerite–protomerite complex concentrically formed by a depressed ovoid epimerite within a transversely oblong protomerite (Fig. 9). The protruding dome of the satellite's epimerite fits into a caudal depression of the primite's deutomerite and the transverse anterior margin of the satellite's protomerite mates with the posterior margin of the primite to form the syzygial junction. Delineation of epimerite and protomerite becomes indistinct with age and maturity in satellites (Figs. 5–8, 13). Although both species possess dolioform oocysts, those of *T. sphinx* (length 5.2 μm, 3.1 width μm) are shorter than those of *T. stammeri* (length 7 μm, width 3 μm [Geus, 1969]).

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