

STYLOCEPHALUS OCCIDENTALIS N. SP. (APICOMPLEXA: EUGREGARINIDA: STYLOCEPHALIDAE) FROM TRIMYTIS PRUINOSA (COLEOPTERA: TENEBRIONIDAE) IN THE NEBRASKA SANDHILLS

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ABSTRACT: *Stylocephalus occidentalis* n. sp. (Apicomplexa: Eugregarinida) is described from *Trimytis pruinosa* (Coleoptera: Tenebrionidae) collected from Keith County in the Sandhills of western Nebraska. Measurements are means in micrometers. Developing trophozoites solitary; epimerite a complex of terminal epimerite and intercalating diamerite; epimerite shallowly ovoid to transversely elliptoid, with transverse basal constriction at junction with diamerite, length 0.5–1 times width, approximately 3–4 times that of diamerite; width approximately equal to that of diamerite; diamerite roughly cylindrical to spindle-shaped, without significant anterior taper, little or no evidence of longitudinal folds, length approximately twice width. Association late, frontal, isogamontic. Gamont protomerite depressed ovoid to very broadly ovoid, length 27.3, width 35.1, anterior distance to widest point 15.4. Protomerite–deutomerite septum clearly marked and constricted, width 34.6. Deutomerite often with distinct marginal crenulation, narrowly obovoid to very narrowly obovoid, length 35.6, maximum width 57.6, anterior distance to widest point 26.3, equatorial width 35.1, ± 12.5 , 29. Total length 381.5. Nucleus ellipsoid, length 32.5, width 18.8; with 0 or 2 polysomal endosomes. Gametocysts roughly spherical; diameter 205.0; wall desiccating to become paper-like, slightly papillated, dehiscing by simple rupture, releasing oocysts in coiled chains, epispore packet absent, gametocyst residuum present. Oocysts dark brown to black, axially asymmetric, broadly deltoid, gibbous in lateral aspect, slightly keeled in dorsal aspect; length 9.8, height 7.9; with slight terminal protuberances and 2 central, spherical residua.

The genus *Stylocephalus* comprises 37 species united by the general form of the diamerite and the epimerite proper (Levine, 1988). The genus is known primarily from the Palearctic and Paleotropics. In the Palearctic, *Stylocephalus* species occur in Europe (Léger, 1904; Foerster, 1938; Grell, 1940; Tuzet and Théodoridès, 1951; Tuzet and Ormières, 1955, 1956; Théodoridès, 1955a, 1960a, 1963a; Lipa, 1967; Corbel, 1971), the Mediterranean (Filipponi, 1949, 1951; Théodoridès, 1955a, 1960b, 1982; Ormières, 1967), the Near East (Théodoridès, 1955a, 1955b, 1955c, 1961), and the Far East (Hoshide, 1951, 1958; Théodoridès et al., 1976). In the Paleotropics, *Stylocephalus* species occur on the Indian subcontinent (Misra, 1941, 1942; Théodoridès, 1966; Devdhar and Amoji, 1977; Haldar and Chakraborty, 1979; Patil and Amoji, 1984) and in Southeast Asia (Théodoridès and Desportes, 1966; Théodoridès et al., 1975, 1984) and Africa (Gibbs, 1946; Théodoridès and Pierre, 1961; Théodoridès and Jolivet, 1963, 1982; Théodoridès et al., 1964, 1965) including Madagascar (Théodoridès, 1959) and the Cape Verde Islands (Théodoridès and Jolivet, 1986). In the Nearctic 7 *Stylocephalus* species occur (Ellis, 1912a, 1913; Nelson, 1970) including the type species, *Stylocephalus giganteus* Ellis, 1912 that was originally described from an undetermined species of *Eleodes* (Coleoptera: Tenebrionidae: Eleodiini) in Colorado (Ellis, 1912a). A single species of *Stylocephalus* is described from the Neotropics (Ellis, 1912b). *Stylocephalus* are known exclusively from tenebrionid beetles and although no stylocephalid has been reported from the Australian or Oceanian regions of the world, neither does the literature indicate a gregarine survey of the tenebrionid beetles in these regions. Thus the distribution of the genus in Australia and Oceania awaits survey and clarification.

During an on-going biotic survey of the gregarine parasites of North American insects, a heretofore unknown gregarine species was discovered in populations of *Trimytis pruinosa* LeConte, 1851 (Insecta: Coleoptera: Tenebrionidae: Tentyri-

inae: Trimytini) in the Sandhills region of western Nebraska. The gregarine populations recovered are taxonomically distinct from known gregarine species and represent a new species of *Stylocephalus* Ellis, 1912. The description presented herein utilizes the extended gregarine morphometric set introduced by Clopton (1999), applies these characters only to mature or invariant stages, further develops a standardized discontinuous epimerite character set for the Stylocephalidae, and proposes a new species within the genus *Stylocephalus*.

MATERIALS AND METHODS

Trimytis pruinosa LeConte, 1851 adults ($n = 69$) were collected from beneath cattle dung pats in the loess hills south of Cedar Point Biological Station (CPBS), approximately 1 km southeast of Lake McConaughy, Keith County, Nebraska between 20 July and 4 August 1998 (Fig. 1). Locality coordinates were determined using an Eagle Explorer Global Positioning Satellite locator. Beetles were transported to the laboratory at CPBS, divided into lots of 4–6 individuals each, and held in 100-ml glass culture dishes (Carolina Culture Dishes, Carolina Biological Supply Company, Burlington, North Carolina) with damp filter paper. All beetles were held for at least 6 hr for gametocyst shedding and then either preserved as permanent specimens or examined for gregarine infection within 48 hr of collection. Beetles were eviscerated and their alimentary canals dissected into insect muscle saline (Belton and Grundfest, 1962). Intestines were blotted to remove excess saline and permanent parasite preparations were made using wet smears of gregarines and host gut tissues (Clopton, 1996, 1999). Smears were fixed for 3 min in AFA (ethanol, formalin, and acetic acid), washed and hardened in 70% ethanol (EtOH) for 5 min, and stained with either Semichon's acetocarmine or Harris hematoxylin and eosin-xylo. Stained specimens were dehydrated in an EtOH series, cleared with xylene, and mounted in Damar balsam.

Gametocysts were extracted from collected feces and transferred into individual wells of a Miniwell® assay plate (Nuncclon Miniwell® mini-tray plate; 60 conical, flat-bottomed, 10- μ l wells; Nuncclon 439225, Nalge Nunc International Corp., Rochester, New York). 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 in agar monolayer mounts (Clopton, 1999).

Observations were made using an Olympus B-Max 50 compound microscope with $\times 20$, $\times 40$, and $\times 100$ universal planapochromatic objectives and either phase-contrast condensers or differential interference contrast prisms. Digital photographs were taken with an AGFA

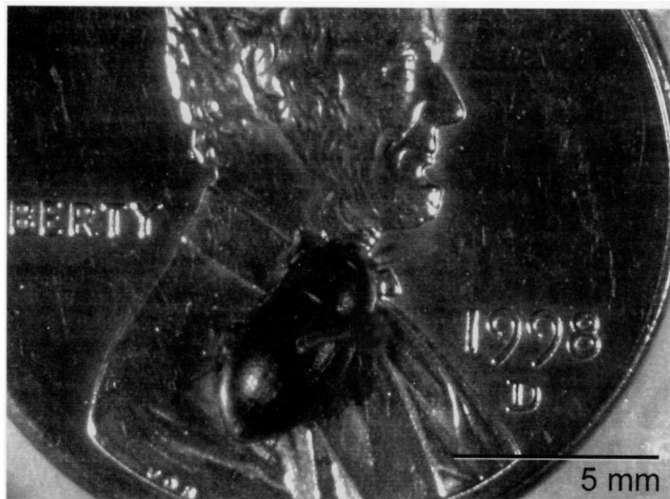


FIGURE 1. *Trimyctis pruinosus* on the head of a penny.

ActionCam digital camera through the aforementioned microscope with either a green density filter (phase-contrast condensers) or a neutral density filter (differential interference contrast prisms and condenser). Measurements were taken from digitized images of live specimens using BioScan Optimas® version 4.1 image analysis software (BioScan Inc., Edmonds, Washington). Drawings were made using digitized images of live and fixed specimens. Photographic plates were processed and assembled using Adobe Photoshop® 4.0 software (Adobe Systems, Inc., San Jose, California).

Morphometric measurements taken were proposed by Clopton (1999). Measurements are presented in μm as range values followed by means, standard deviations, and sample sizes in parentheses. Terminology for parasite ontogenetic stages and anatomy largely follows that proposed by Levine (1971). Filipponi (1949) recognized 2 distinct components comprising an epimerite complex in *Stylocephalus*; the terminal epimerite or holdfast proper, and the diamerite, an intercalating neck or stalk between the epimerite and the protomerite. Filipponi's (1949) epimerite complex terminology is adopted herein. Terminology for shapes of planes and solids is consistent with that suggested by the Systematics Association Committee for Descriptive Biological Terminology (Anonymous, 1962). Additional terminology is derived from Harris and Harris (1994).

DESCRIPTION

Diagnosis

Eugregarinida Léger, 1892 sensu strictu Levine et al. (1980); Septatina Lankester, 1885, sensu strictu Levine et al. (1980); Stenophoricae Levine, 1984 sensu Chakravarty, 1960; Stylocephalidae Ellis, 1912; with the characters of the genus *Stylocephalus* Ellis, 1912 sensu Théodoridès, 1963b: epimerite complex elongated into a cylindrical diamerite, expanding terminally to form the epimerite proper; epimerite a simple, spherical button; gametocysts papillate, with internal pseudocyst residuum; oocysts axially asymmetric, broadly deltoid, gibbous in lateral aspect, slightly keeled in dorsal aspect (including hat-, purse-, stone-, and seed-shaped of previous authors), emerging in chains.

Stylocephalus occidentalis n. sp.

(Figs. 2–21)

Trophozoite (Figs. 2, 3, 8–13): Developing trophozoites solitary, attached to host ventricular epithelium. Holdfast an epimerite complex of terminal epimerite and intercalating diamerite. Epimerite shallowly ovoid to transversely ellipsoid, with transverse basal constriction at junction with diamerite, length 0.5–1 times width, approximately 3–4 times that of diamerite; width approximately equal to that of diamerite; diamerite roughly cylindrical to spindle-shaped, without significant anterior taper, little or no evidence of longitudinal folds, length approximately twice width; without visible septum at junction with protomerite but

clearly differentiated by decreased density of cytoplasm. Protomerite transversely ovoid to shallowly ovoid. Protomerite–deutomerite septum clearly marked and constricted. Deutomerite narrowly obovoid to very narrowly obovoid. Nucleus ellipsoid; with a variable number of polysomal endosomes.

Gamont (Figs. 4, 5, 14–17): Protomerite depressed ovoid to very broadly ovoid, length (PL) 17.2–39.6 (27.3, ± 5.2 , 29), width (PW) 21.5–51.6 (35.1, ± 8.7 , 29), PL/PW 0.6–1.0 (0.8, ± 0.1 , 29), anterior distance to widest point (PLA) 8.4–27.9 (15.4, ± 4.4 , 29), posterior distance to widest point (PLP) 5.6–22.8 (12.4, ± 3.2 , 29), PLA/PLP 0.7–2.4 (1.3, ± 0.5 , 29), PLA/PW 0.3–0.7 (0.5, ± 0.1 , 29), PLP/PW 0.2–0.6 (0.4, ± 0.1 , 29). Protomerite–deutomerite septum clearly marked and constricted, width (SW) 21–50.6 (34.6, ± 9.1 , 29), PW/SW 0.9–1.2 (1.0, ± 0.1 , 29). Deutomerite often with distinct marginal crenulation or superficial annulation (Figs. 4, 14, 15), narrowly obovoid to very narrowly obovoid, length (DL) 166.5–608.1 (356.5, ± 107.0 , 29), maximum width (DWM) 32.1–111.3 (57.6, ± 23.4 , 29), anterior distance to widest point (DLA) 11.0–80.3 (26.3, ± 15.0 , 29), posterior distance to widest point (DLP) 145.6–572.4 (331.5, ± 104.3 , 29), equatorial width (DWE) 18.7–62.1 (35.1, ± 12.5 , 29), anterior distance to equatorial plane (DLH) 85.6–304.5 (180.1, ± 52.4 , 29), DL/DWM 3.8–10.1 (6.6, ± 1.7 , 29), DWM/DWE 1.3–2.6 (1.6, ± 0.3 , 29), DLA/DWM 0.3–1.3 (0.5, ± 0.2 , 29), DLP/DWM 3.3–9.9 (6.1, ± 1.7 , 29), DLH/DWE 2.8–10.6 (5.4, ± 1.6 , 29), DWM/SW 1.2–2.3 (1.6, ± 0.3 , 29), DWE/SW 0.7–1.3 (1.0, ± 0.2 , 29). Total length (TL) 186.4–639.6 (381.5, ± 110.5 , 29). Indices: TL/PL 7.7–23.8 (14.1, ± 3.7 , 29), DL/PL 6.9–22.8 (13.2, ± 3.7 , 29), DWM/PW 1.2–2.5 (1.6, ± 0.3 , 29), DWE/PW 0.6–1.4 (1.0, ± 0.2 , 29), TL/DL 1.0–1.1 (1.1, ± 0 , 29). Nucleus ellipsoid, typically abaxial; length (NL) 13.9–42.7 (32.5, ± 6.5 , 25), width (NW) 8.5–25.8 (18.8, ± 4.8 , 25), distance to protomerite–deutomerite septum (NDS) 6.3–143.2 (45.4, ± 38.6 , 25), NL/NW 1.3–2.9 (1.8, ± 0.4 , 25); with 0 or 2 polysomal endosomes, diameter (KD) 7.7–16.8 (10.6, ± 2.7 , 13).

Association: Frontal; isogamontic; late and ephemeral; leading directly to syzygy, associated pairs fusing laterally during syzygy; associations, syzygial pairs, and gametocysts located between host ventricular peritrophic membrane and posterior ventricular epithelium. Gamonts in association morphometrically similar to solitary gamonts; epimerite absent.

Gametocysts (Figs. 18–20): White to opalescent in color (Fig. 18), becoming dark brown to black with maturity (Fig. 19); roughly spherical; diameter 179.0–233.0 (205.0, ± 18.5 , 29); no hyaline coat apparent, gametocyst wall desiccating to become paper-like and slightly papillated. Gametocysts mature within 48–72 hr and dehisce by simple rupture of the gametocyst walls. Oocysts are extruded in a coiled chain to form a single, tangled, sticky mass (Fig. 20); epispore packet absent, gametocyst residuum present.

Oocysts (Figs. 6, 7, 20, 21): Axially asymmetric, broadly deltoid, gibbous in lateral aspect, slightly keeled in dorsal aspect, very uniform in size and shape; length (OL) 9–10.3 (9.8, ± 0.3 , 41); height (OH) 7.3–8.7 (7.9, ± 0.3 , 41); with slight terminal protuberances or shoulders, height (SH) 1.3–2.5 (1.8, ± 0.3 , 41); with 2 central, spherical residua, diameter residuum 1 1.6–2.5 (2.0, ± 0.3 , 26), diameter residuum 2 1.6–2.8 (2, ± 0.3 , 22); octozooic, sporozoites resting in tandem, folded around central residuum. Extruded in chains (Fig. 20). Oocysts dark brown under transmitted light, black under reflected light.

Taxonomic summary

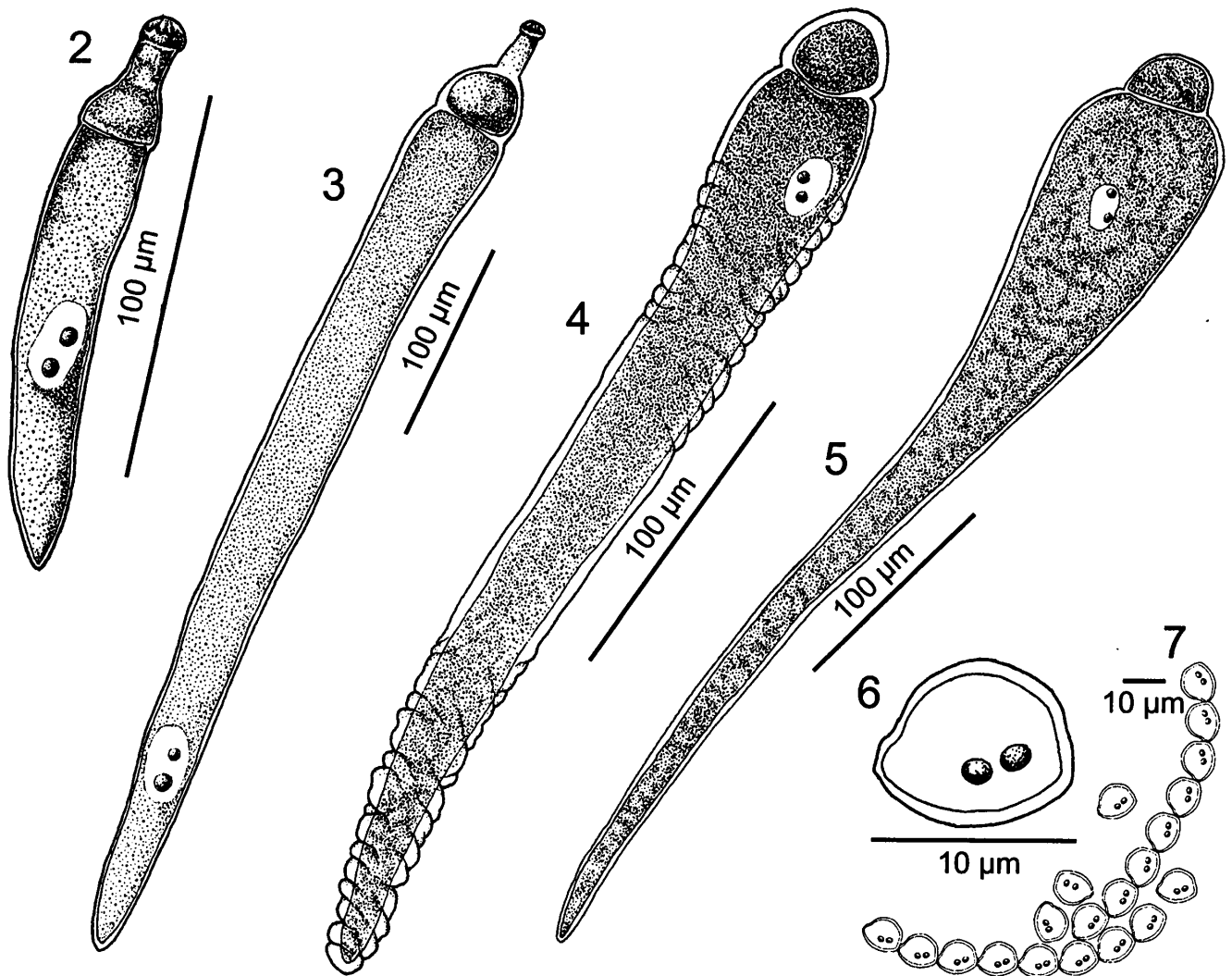
Host: *Trimyctis pruinosus* LeConte, 1851 (Insecta: Coleoptera: Tenebrionidae: Tentyriinae: Trimytini).

Symbiotype: One symbiotype specimen is deposited with the Division of Entomology, University of Nebraska State Museum (UNSM), Lincoln, Nebraska. The symbiotype is identified with 3 labels: a collection label, "NE: Keith Co.; Cedar Point Biol Stn, N41°12'25.8", W101°36'56.8", 20 July 1998: R. E. Clopton"; an NSF Deposition label, "Clopton: NSF DEB-9705179, NSF PROJECT VOUCHER, REC-980249"; and a blue UNSM voucher label "RESEARCH PROJECT Voucher Specimen." Additional voucher specimens (REC980250–REC980253 and REC980333, REC980334) are retained by the author.

Host records: *Trimyctis pruinosus*; adults.

Locality: N41°12'25.8", W101°36'56.8", CPBS, 1 km southeast of Lake McConaughy, Keith County, Nebraska.

Infection site: Trophozoites and gamonts were observed along the length of the ventriculus, anterior to the ileum and the attachment of



FIGURES 2–7. *Stylocephalus occidentalis*, n. sp. 2. Young trophozoite. 3. Older trophozoite. 4. Young gamont, note superficial crenulation. 5. Mature gamont. 6. Oocyst with residua. 7. Oocyst chains.

the Malpighian tubules. Associations primarily located in the ileum. All endogenous life-cycle stages were observed between host ventricular peritrophic membrane and ventricular epithelium. Gametocysts collected from host feces.

Prevalence: 91.3% (63 of 69 beetles examined post mortem).

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 a trophozoite on slide HWML 15005 (author's slide REC980476) and is marked by an etched circle. The remaining trophozoites, gamonts, and associations in HWML 15006 (8 slides, author's slides REC980254–REC980257, REC980260, REC980263, REC980264, REC98267) are paratypes. Trophozoites, gamonts, and associations on author's slides REC980269, REC980271, REC980274, REC980276a–b, REC980295, REC980298–REC980300, REC980477a–b, and REC980480–REC980482 are paratypes retained by the author.

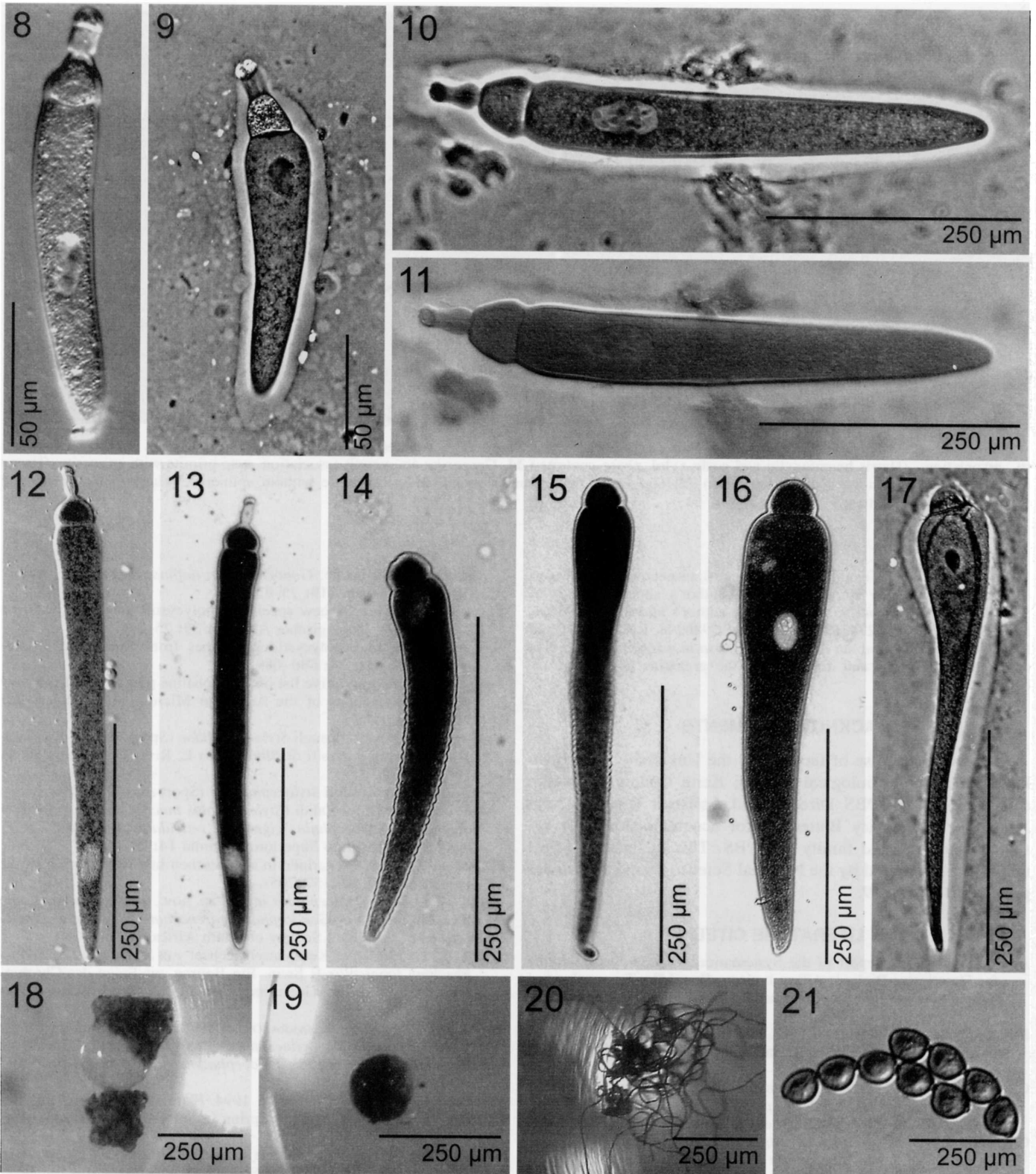
Erymology: The specific epithet *occidentalis* (Latin, “of the west”) is given to mark the New World distribution of this species.

Remarks

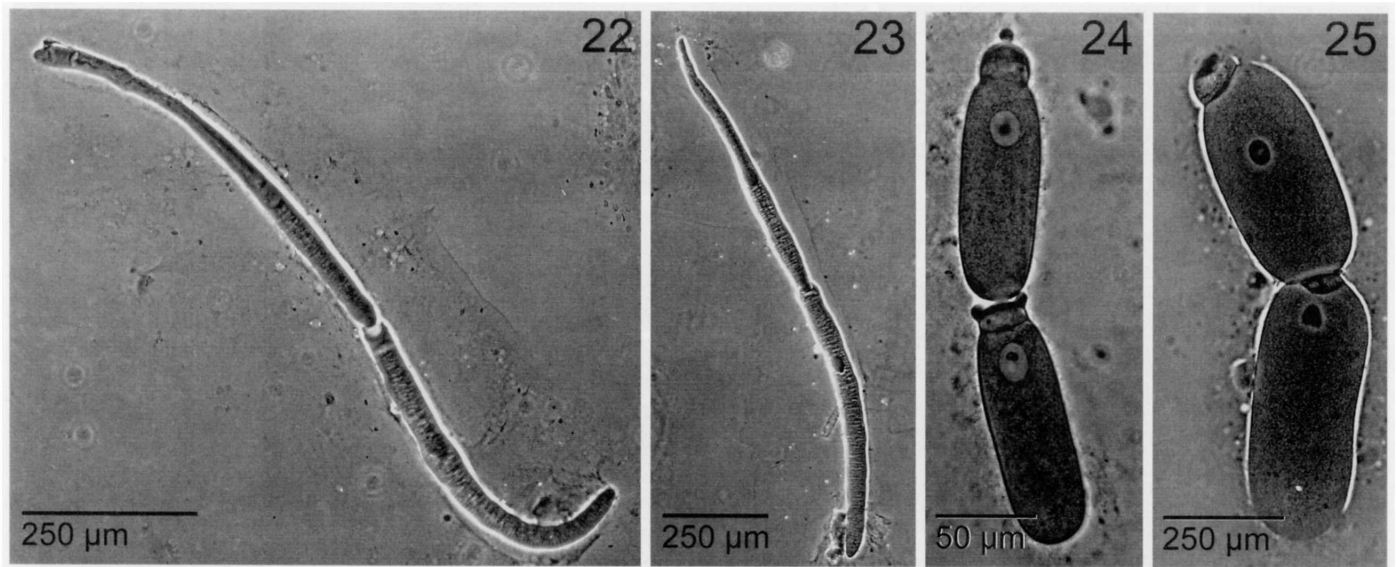
Stylocephalus occidentalis is the first species of *Stylocephalus* described from the New World Tentyriinae. This gregarine is distinguished from most species in the genus *Stylocephalus* by overt differences in

the form of the epimerite proper. The ovoid epimerite proper of *S. occidentalis* is shared by only 3 other members of the genus: *Stylocephalus coniontis* Nelson, 1970, *Stylocephalus filiformis* Théodoridès, 1959, and *Stylocephalus pauliani* Théodoridès, 1959, but these taxa are readily distinguished. Gamonts of *S. occidentalis* range 186.4–639.6, their average length (381.5) less than one-third that reported for *S. coniontis* (1,105 [Nelson, 1970]) and less than one-fourth that reported for *S. filiformis* and *S. pauliani* (1,700 and 1,800, respectively [Théodoridès, 1959]). *Stylocephalus occidentalis* is further differentiated from *S. pauliani* by differences in oocyst size that measure 9.7×8 and 17×12.5 , respectively (Théodoridès, 1959). The oocysts of *S. coniontis* and *S. filiformis* are unknown.

Two other gregarine taxa were recovered from *T. pruinosa* but neither in numbers sufficient to permit accurate description nor diagnosis beyond the generic level. Individuals of *Protomagalhaensia* sp. were readily diagnosed by the long, filiform nature of the gamont and the interlocking structure of the primite–satellite junction (Figs. 22, 23). Individuals of *Gregarina* sp. were provisionally diagnosed based on the general structure of the gamont, the form of the association, and distinct spherical epimerite (Figs. 24, 25). A more complete diagnosis of this taxon is impossible without data on gametocysts and oocysts that were not recovered. Observed prevalences of *Protomagalhaensia* sp. and *Gregarina* sp. were 1.4% (1 of 69 beetles examined post mortem) and



FIGURES 8–21. *Stylocephalus occidentalis*, n. sp. 8. Young trophozoite. (Live, differential interference contrast.) 9. Young trophozoite. (Harris hematoxylin and eosin-xylo, phase contrast.) 10. Holotype, an older trophozoite. (Harris hematoxylin and eosin-xylo, phase contrast.) 11. Holotype, an older trophozoite. (Harris hematoxylin and eosin-xylo, differential interference contrast.) 12. Older trophozoite. (Live, differential interference contrast.) 13. Older trophozoite. (Live, brightfield.) 14. Young gamont, note superficial crenulation. (Live, brightfield.) 15. Older gamont, note superficial crenulation. (Live, brightfield.) 16. Mature gamont without superficial crenulation. (Harris hematoxylin and eosin-xylo, phase contrast.) 17. Mature gamont without superficial crenulation. (Harris hematoxylin and eosin-xylo, phase contrast.) 18. Freshly collected gametocyst with attached host fecal material, 6 hr. 19. Gametocyst with dark, micropapillated epicyst wall, 24 hr. 20. Gametocyst dehiscence, 72 hr. 21. Chains of oocysts. (Agar oocyst monolayer, differential interference contrast.)



FIGURES 22–25. Gregarines of the genera *Protomagalhaensia* and *Gregarina* recovered from *Trimytis pruinosa*. 22, 23. *Protomagalhaensia* sp., associations. (Harris hematoxylin and eosin-xytol, phase contrast.) 24. *Gregarina* sp., young association, note primitive with epimerite. (Harris hematoxylin and eosin-xytol, phase contrast.) 25. *Gregarina* sp., mature association, note primitive without epimerite. (Harris hematoxylin and eosin-xytol, phase contrast.)

11.6% (8 of 69 beetles examined post mortem), respectively. Specimens of *Protomagalhaensia* sp. are found on author's slide REC980479. Specimens of *Gregarina* sp. are found on author's slides REC980261, REC980269, REC980274, REC980275, REC980298, REC980480, and REC980481. Additional survey of *T. pruinosa* is warranted to provide complete descriptions and diagnoses of the gregarine assemblage inhabiting this beetle.

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