Trichurispora wellgundis n. g., n. sp. (Apicomplexa: Eugregarinida: Hirmocystidae) Parasitizing Adult Water Scavenger Beetles, *Tropisternus collaris* (Coleoptera: Hydrophilidae) in the Texas Big Thicket

R. E. Clopton,[1,3] T. J. Cook,[2] and J. L. Cook[2]

[1] Department of Natural Science, Peru State College, Peru, Nebraska, U.S.A. and

[2] Department of Biological Sciences, Sam Houston State University, Huntsville, Texas 77341-2166, U.S.A.

ABSTRACT: *Trichurispora wellgundis* n. g., n. sp. (Apicomplexa: Eugregarinida: Hirmocystidae) is described from the adults of the water scavenger beetle *Tropisternus collaris* (Coleoptera: Hydrophilidae) collected from B A Steinhagen Lake in the Cherokee Unit of the Big Thicket National Preserve, Tyler County, Texas, U.S.A. *Trichurispora* is distinguished from known genera of Hirmocystidae by a distinct "trichurisiform" oocyst that is hesperidiform in outline, comprising a fusiform oocyst with shallowly ovoid terminal knobs or caps. Oocyst residua are present but confined to a central fusiform residuum vacuole. Adult and larval hydrophilid beetles represent distinctly different opportunities for parasite colonization and diversification. Gregarines have been reported from both adult and larval hydrophilid beetles, but no species and no genus is reported from both adult and larval hosts. In fact, gregarine taxic richness is often more disparate between adult and larval beetles of the same species than between host beetle species. This is the first report of a septate gregarine from an adult hydrophilid beetle in the Nearctic.

KEY WORDS: Apicomplexa, Eugregarinida, Hirmocystidae, Gregarine, *Trichurispora wellgundis* n. g., n. sp., *Didymophyes*, *Enterocystis hydrophili* incertae sedis, *Stylocephalus brevirostris* incertae sedis, Coleoptera, Hydrophilidae, *Tropisternus collaris*, Texas, Big Thicket, U.S.A.

As part of an ongoing survey of the insect and eugregarine diversity of the Primitive Big Thicket region of east-central Texas, U.S.A., we collected an heretofore unknown gregarine species from adults of water-scavenger beetles, *Tropisternus collaris* (Coleoptera: Hydrophilidae). The gregarines recovered are referable to the family Hirmocystidae but are taxonomically distinct from all existing genera within the family. Herein we recognize and describe *Trichurispora* n. gen. within Hirmocystidae, delineate an extended gregarine morphometric set for *Trichurispora*, describe the new taxon as the type species, recognize the probable congeneric status of a previously described gregarine from hydrophilid beetles in France, and hypothesize likely trends in diversification and host colonization in gregarines parasitizing hydrophilid beetles.

MATERIALS AND METHODS

Collection, preservation, and analysis of specimens

On September 28, 2006, 134 adults of *T. collaris* were collected from Town Bluff Slough of B A Steinhagen Lake, in the western parcel of Martin Dies Jr. State Park, Cherokee Unit, Big Thicket National Preserve, Tyler County, Texas, U.S.A. (30°33′06.2″N; 94°41′83.7″W) using aquatic dip

nets, placed in 1-liter plastic jars with pond water and stored on ice for transportation to the laboratory at Sam Houston State University, Huntsville, Texas, U.S.A. Seventy-four beetles were examined for gregarine infection; the remainder were prepared as permanent preparations to serve as host voucher specimens. Beetles were eviscerated and their alimentary canals dissected in dilute insect muscle saline (Belton and Grundfest, 1962). Permanent parasite preparations were made with the use of wet smears of gregarines and host gut tissues fixed by flotation on hot AFA (ethanol, formalin, and acetic acid), stained with either Semichon's acetocarmine or Harris' hematoxylin and eosin–xylol, dehydrated in ethanol series, cleared in xylene series, and mounted in Damar balsam. Subsamples of gregarines from 15 hosts were collected and pooled in groups of 30 individuals each. The DNA from each pooled sample was isolated with the use of a protocol similar to that reported by Laird et al. (1991). Isolated DNA samples were resuspended in AE buffer (10 mM Tris Cl; 0.5 mM EDTA, pH 9.0) and stored at −20°C for future genetic analysis.

Gametocysts were isolated from the alimentary tracts of host beetles during dissection, triple-rinsed in insect muscle saline, surface sterilized in 1% formalin, triple-rinsed in spring water, and placed in individual silicon-stoppered 4 × 12-mm glass microvials (BioQuip Products, Gardena, California, U.S.A.) with ca. 50 μl spring water and held for maturation and dehiscence. Gametocysts were observed daily and any changes in structure, maturation or dehiscence noted. Oocyst structure and dimensions were taken from fresh preparations of oocysts in wet mounts and agar monolayer mounts prepared as follows. Molten agar (1.5% solution) was pipetted onto a clean glass slide and allowed to drain, and the slide was quickly chilled on a cold aluminum block to produce a thin, uniform layer of agar. Oocysts were placed in a small (ca. 7 μl) drop of water on a 12-mm round cover glass (#0 thickness). The agar slide was inverted to

[3] Corresponding author (e-mail: rclopton@oakmail.peru.edu).
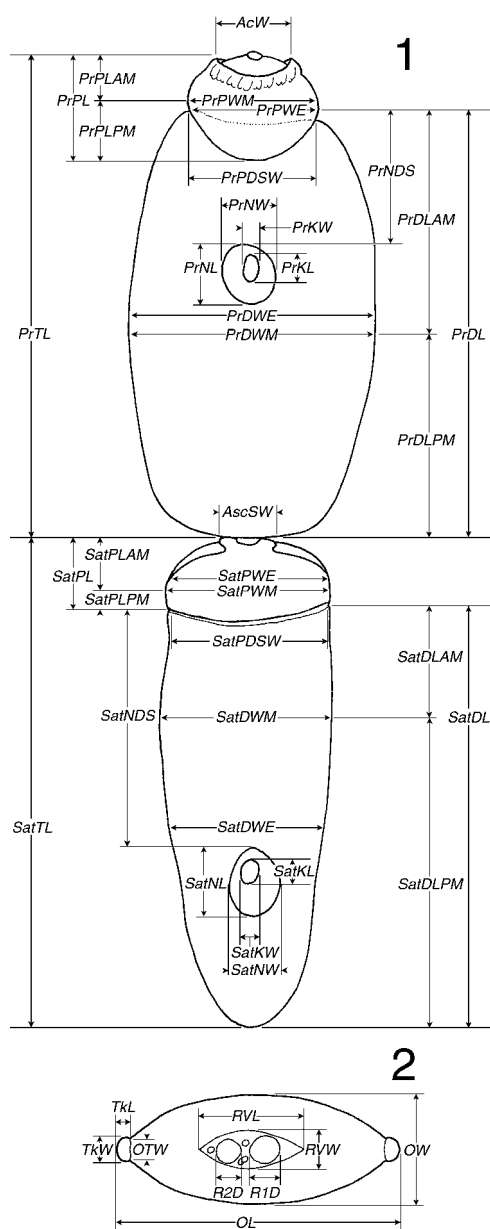
Figures 1, 2. Morphometric character set for gamonts and oocysts of *Trichurispora* species. **1.** Gamont characters mapped on a mature association of *Trichurispora wellgundis* n. sp. **2.** Oocyst characters mapped onto oocyst of *Trichurispora wellgundis* n. sp. (AcW, acetabulum width; AscSW, width of primite–satellite junction; OL, oocyst length; OTW, oocyst terminal width; OW, oocyst width; PrDL, length of primite deutomerite; PrDLAM, distance from primite protomerite–deutomerite septum to deutomerite axis of maximum width; PrDLPM, distance from posterior end of primite deutomerite to deutomerite axis of maximum width; PrDWE, width of primite deutomerite at equatorial axis;

pick up the cover glass. The resulting preparation produced a monolayer of oocysts trapped between the agar layer and the cover glass, providing a uniform object plane for light microscopy.

Observations were made using an Olympus B-Max 50 compound microscope with ×10, ×20, ×40, and ×60 universal planapochromatic objectives with either phase contrast condensers or differential interference contrast prisms and an infinity-optics turret doubler. Digital photographs were taken with an Olympus DP-70 digital camera through the aforementioned microscope. Measurements were taken from the digitized images of preserved specimens using Image-Pro Discovery® v 4.0 image analysis software (Media Cybernetics, L.P., Silver Spring, Maryland, U.S.A.). Photographic plates were processed and assembled using Adobe PhotoShop® 7.0.1 software (Adobe Systems Inc., San Jose, California, U.S.A.).

Morphometric characters and abbreviations

Extended gregarine morphometric sets (e.g., Clopton, 2004a, 2006; Clopton et al., 2004; Clopton and Hays, 2006) include both standard mensural data and ratios common to all gregarine species and additional metrics particular to the genus of study. The extended character set used herein for *Trichurispora* is delineated in Figures 1 and 2. Measurements are presented in micrometers as mean values followed

←

PrDWM, maximum width of primite deutomerite; PrKL, primite karyosome length; PrKW, primite karyosome width; PrNDS, distance from anterior margin of primite nucleus to protomerite–deutomerite septum; PrNL, length of primite nucleus; PrNW, width of primite nucleus; PrPDSW, width of primite's protomerite–deutomerite septum; PrPL, length of primite protomerite; PrPLAM, distance from anterior end of primite to protomerite axis of maximum width; PrPLPM, distance from primite's protomerite–deutomerite septum to protomerite axis of maximum width; PrPWE, width of primite protomerite at equatorial axis; PrPWM, maximum width of primite protomerite; PrTL, total length of primite; R1D, diameter of primary residuum; R2D, diameter of secondary residuum; RVL, length of residuum vacuole; RVW, width of residuum vacuole; SatDL, length of satellite deutomerite; SatDLAM, distance from satellite's protomerite–deutomerite septum to deutomerite axis of maximum width; SatDLPM, distance from posterior end of satellite deutomerite to deutomerite axis of maximum width; SatDWE, width of satellite deutomerite at equatorial axis; SatDWM, maximum width of satellite deutomerite; SatKL, satellite karyosome length; SatKW, satellite karyosome width; SatNDS, distance from anterior margin of satellite nucleus to protomerite–deutomerite septum; SatNL, length of satellite nucleus; SatNW, width of satellite nucleus; SatPDSW, width of satellite's protomerite–deutomerite septum; SatPL, length of satellite protomerite; SatPLAM, distance from anterior end of satellite protomerite to protomerite axis of maximum width; SatPLPM, distance from satellite's protomerite–deutomerite septum to protomerite axis of maximum width; SatPWE, width of satellite protomerite at equatorial axis; SatPWM, maximum width of satellite protomerite; SatTL, total length of satellite; TkL, length of terminal oocyst knob; TkW, width of terminal oocyst knob).

by range values, standard deviations, and sample sizes in parentheses. Terminology for parasite ontogenetic stages and anatomy generally follows that proposed by Levine (1971). Terminology for shapes of planes and solids follows Clopton (2004b). Additional descriptive terminology is derived from Harris and Harris (1994).

The following metric characters and abbreviations are used herein: acetabulum width (AcW), width of primite–satellite junction (AscSW), oocyst length (OL), oocyst terminal width (OTW), oocyst width (OW), length of primite's deutomerite (PrDL), distance from primite's protomerite–deutomerite septum to deutomerite's axis of maximum width (PrDLAM), distance from posterior end of primite's deutomerite to deutomerite's axis of maximum width (PrDLPM), width of primite's deutomerite at equatorial axis (PrDWE), maximum width of primite's deutomerite (PrDWM), primite's karyosome length (PrKL), primite's karyosome width (PrKW), distance from anterior margin of primite's nucleus to protomerite–deutomerite septum (PrNDS), length of primite's nucleus (PrNL), width of primite's nucleus (PrNW), width of primite's protomerite–deutomerite septum (PrPDSW), length of primite's protomerite (PrPL), distance from anterior end of primite to protomerite's axis of maximum width (PrPLAM), distance from primite's protomerite–deutomerite septum to protomerite's axis of maximum width (PrPLPM), width of primite's protomerite at equatorial axis (PrPWE), maximum width of primite's protomerite (PrPWM), total length of primite (PrTL), diameter of primary residuum (R1D), diameter of secondary residuum (R2D), length of residuum vacuole (RVL), width of residuum vacuole (RVW), length of satellite's deutomerite (SatDL), distance from satellite's protomerite–deutomerite septum to deutomerite's axis of maximum width (SatDLAM), distance from posterior end of satellite's deutomerite to deutomerite's axis of maximum width (SatDLPM), width of satellite's deutomerite at equatorial axis (SatDWE), maximum width of satellite's deutomerite (SatDWM), satellite's karyosome length (SatKL), satellite's karyosome width (SatKW), distance from anterior margin of satellite's nucleus to protomerite–deutomerite septum (SatNDS), length of satellite's nucleus (SatNL), width of satellite's nucleus (SatNW), width of satellite's protomerite–deutomerite septum (SatPDSW), length of satellite's protomerite (SatPL), distance from anterior end of satellite's protomerite to protomerite's axis of maximum width (SatPLAM), distance from satellite's protomerite–deutomerite septum to protomerite's axis of maximum width (SatPLPM), width of satellite's protomerite at equatorial axis (SatPWE), maximum width of satellite's protomerite (SatPWM), total length of satellite (SatTL), length of terminal oocyst knob (TkL), and width of terminal oocyst knob (TkW).

Use of species inquirenda, incertae sedis, and lapsus calami

We adopt the following guidelines for the use of "species inquirenda," "incertae sedis," and "lapsus calami" at the species level in gregarine taxonomy and systematics. Species inquirendae are gregarine taxa that can be confidently referred to an existing genus but for which existing data sets are insufficient to assess all of the cardinal characters that distinguish species within the genus confidently. Such taxa require new collections and redescription to establish and stabilize their identity fully. A gregarine taxon is of incertae sedis if it cannot be confidently referred to an existing genus because of insufficient data, distinguished as an indicator taxon representing a new genus based on the presence of unique characters, or confidently synonymized with existing stable taxa. These are, in essence, taxa that are unique but so poorly defined that they probably would not be recognized on recollection. We deem lapsus calami all subsequent incorrect spellings unless the author made specific their intent to emend the spelling of a name. Names formed by lapsus calami have no nomenclatural status.

RESULTS

Trichurispora n. gen.

Diagnosis

With the characters of Order Eugregarinorida Léger, 1892, sensu Clopton (2002); Suborder Septatorina Lankester, 1885, sensu Clopton (2002); Superfamily Gregarinicae Chakaravarty, 1960 sensu Clopton (2002); Family Hirmocystidae Grassé, 1953, sensu Clopton (2002); and characters of the genus *Trichurispora* n. gen. as follows: association precocious, caudofrontal; epimerite in the form of a complex including an apical orbicular tumidus within an acetabulum formed by extension of the anterior protomerite; oocysts hesperidiform in outline, comprising a fusiform oocyst with shallowly ovoid terminal knobs or caps; oocyst residua present, generally confined to a central fusiform residuum vacuole, released from gametocysts by simple rupture.

Taxonomic summary

Type species: Trichurispora wellgundis n. sp.

Etymology: The name *Trichurispora* reflects the overall resemblance between the oocysts of this taxon and the eggs of trichurid nematodes, particularly the human whipworm, *Trichuris trichiura*.

Remarks

Clopton and Hays (2006) reviewed and evaluated the cardinal characters of genera comprising Hirmocystidae. Genera within the family often are distinguished with the use of a suite of characters, including proclivity for multiple associations; oocyst shape; shape and coronate nature of the epimerite; and shape and polarity of the association interface. However, *Trichurispora* is distinguished within the family by oocyst shape alone. Among the genera comprising Hirmocystidae, only species of *Trichurispora* possess fusiform oocysts with shallowly ovoid terminal knobs or caps. The oocysts of *Trichurispora*
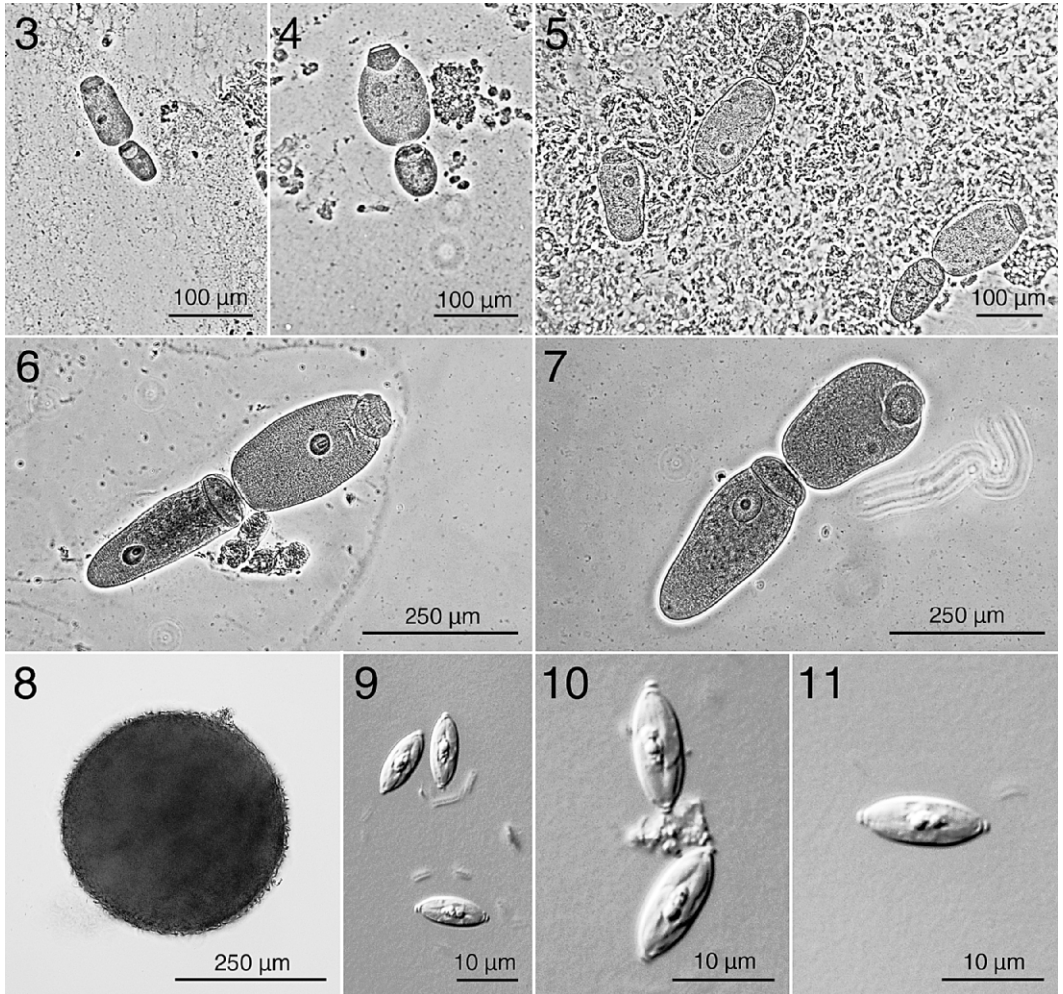
Figures 3–11. Associations and oocysts of *Trichurispora wellgundis* n. sp. **3–5.** Precocious associations of trophozoites, phase contrast microscopy. **6.** Mature gamonts in association, phase contrast microscopy (Holotype). **7.** Mature gamonts in association, phase contrast microscopy. **8.** mature gametocyst with surface halo of oocysts, brightfield microscopy. **9–11.** Oocysts, differential interference contrast microscopy.

species are more similar to those characteristic of *Quadruhalyodiscus* Kundu and Haldar, 1984 than any other member of Hirmocystidae, but the fusiform oocysts of *Quadruhalyodiscus* lack the terminal knobs or caps characteristic of *Trichurispora* oocysts (Kundu and Haldar, 1984). *Trichurispora* and *Quadruhalyodiscus* are distinguished further by epimerite shape: an epimerite complex comprising an apical orbicular tumidus within an acetabulum formed by extension of the anterior protomerite in *Trichurispora* contrasting a diamerite ending in 4 hyaline loculi in *Quadruhalyodiscus* (Kundu and Haldar, 1984).

Trichurispora wellgundis n. sp.
R. E. Clopton and T. J. Cook
(Figs. 1–11)

Description

Trophozoites: Young trophozoites in precocious caudofrontal association, no solitary trophozoite was observed.

Association: Gamonts anisomorphic, epimerite complex retained in mature primites; association precocious, caudofrontal: AscSW 33 (19.5–70.2, ±9.08, 47). Indices: SatNL/SatNW 1.2 (0.8–1.8,

±0.19, 47), SatNDS/SatNL 2.4 (0.5–4.7, ±0.94, 47), SatDL/SatNDS 2.9 (1.4–12.2, ±2.09, 47), SatKL/SatKW 1.2 (0.8–2.6, ±0.31, 47), SatNL/SatKL 2.1 (1.5–2.9, ±0.39, 47), SatPL/PrPL 0.8 (0.4–1.4, ±0.2, 47), SatPWE/PrPWE 1.3 (0.9–1.8, ±0.21, 47), SatPWM/PrPWM 1.4 (0.9–1.9, ±0.23, 47), SatDL/PrDL 0.9 (0.7–1.1, ±0.12, 47), SatDWE/PrDWE 0.8 (0.5–1.5, ±0.16, 47), SatDWM/PrDWM 0.9 (0.6–1.3, ±0.15, 47), SatTL/PrTL 0.9 (0.7–1.1, ±0.12, 47).

Primite: Total length (PrTL) 222.7 (153–300, ±39.05, 47). Epimerite a complex including an apical orbicular tumidus within an acetabulum formed by extension of the anterior protomerite: AcW 34.2 (19.3–47.9, ±6.64, 47). Protomerite shallowly elliptoid to orbicular panduriform: PrPL 46.5 (24–61.1, ±7.79, 47), PrPWE 56.9 (42.3–74.3, ±7.23, 47), PrPWM 59.8 (44.9–78.8, ±6.77, 47), PrPLAM 23.7 (16–30.3, ±3.58, 47), PrPLPM 22 (4.6–35.3, ±6.46, 47), PrPDSW 61.9 (46.6–78.5, ±6.57, 47), PrPL/PrPWE 0.8 (0.5–1, ±0.1, 47), PrPL/PrPWM 0.8 (0.4–1, ±0.1, 47), PrPL/PrPDSW 0.8 (0.4–0.9, ±0.09, 47), PrPLAM/PrPL 0.5 (0.4–0.7, ±0.07, 47), PrPLAM/PrPLPM 1.2 (0.5–5.6, ±0.78, 47), PrPWM/PrPWE 1.1 (1–1.2, ±0.04, 47), PrPWM/PrPDSW 1 (0.8–1, ±0.04, 47). Deutomerite broadly dolioform to dolioform, strongly constricted at protomerite–deutomerite septum: PrDL 194.4 (134–265, ±35.42, 47), PrDWE 111.1 (76.4–153, ±19.21, 47), PrDWM 116.1 (83.1–166, ±20.66, 47), PrDLAM 88.1 (49.8–127, ±19.49, 47), PrDLPM 107.4 (47–184, ±25.16, 47), PrDL/PrDWE 1.8 (1.3–2.5, ±0.24, 47), PrDL/PrDWM 1.7 (1.2–2.2, ±0.19, 47), PrDL/PrPDSW 3.1 (2.2–4, ±0.43, 47), PrDLAM/PrDL 0.5 (0.3–0.7, ±0.08, 47), PrDLAM/PrDLPM 0.9 (0.4–2.3, ±0.34, 47), PrDWM/PrDWE 1 (1–1.3, ±0.06, 47), PrDWM/PrPDSW 1.9 (1.5–2.4, ±0.2, 47). Nucleus roughly orbicular with a single, eccentrically placed orbicular to elliptoid karyosome: PrNL 29.8 (19.4–44.9, ±6.73, 47), PrNW 28 (17.1–55.5, ±7.66, 47), PrNDS 66.7 (13.3–135, ±22.67, 47), PrKL 15.1 (9.2–21.3, ±3.16, 47), PrKW 12.8 (8.2–21.8, ±2.91, 47), PrNL/PrNW 1.1 (0.8–1.5, ±0.17, 47), PrNDS/PrNL 2.3 (0.7–4.6, ±0.82, 47), PrDL/PrNDS 3.3 (1.4–10.1, ±1.56, 47), PrKL/PrKW 1.2 (0.9–1.8, ±0.22, 47), PrNL/PrKL 2 (1.3–3.1, ±0.4, 47). Indices: PrTL/PrPL 4.8 (3.6–7.9, ±0.79, 47), PrDL/PrPL 4.2 (2.8–7, ±0.72, 47), PrDWM/PrPWM 1.9 (1.5–2.6, ±0.21, 47), PrTL/PrDL 1.1 (1.1–1.3, ±0.03, 47).

Satellite: Total length (SatTL) 210.2 (106–313, ±52.71, 47). Protomerite depressed ovoid to broadly ovoid, narrowly joined to primite at association junction, association interface linear, unbiased: SatPL 35.5 (20.5–48.4, ±7.68, 47), SatPWE 75.2 (45.1–103, ±16.25, 47), SatPWM 80.7 (51.3–106, ±15.76, 47), SatPLAM 23 (13.8–34.6, ±5.18, 47), SatPLPM 12.1 (2.6–24.2, ±5.03, 47), SatPDSW 80.8 (51.5–106, ±15.16, 47), SatPL/SatPWE 0.5 (0.2–0.8, ±0.11, 47), SatPL/SatPWM 0.4 (0.2–0.8, ±0.1, 47), SatPL/SatPDSW 0.4 (0.2–0.8, ±0.1, 47), SatPLAM/SatPL 0.7 (0.4–1.2, ±0.13, 47), SatPLAM/SatPLPM 2.4 (0.7–11, ±1.64, 47), SatPWM/SatPWE 1.1 (1–1.3, ±0.05, 47), SatPWM/SatPDSW 1 (0.9–1.1, ±0.03, 47). Deutomerite obovoid, without deep constriction at protomerite–deutomerite septum: SatDL 179.1 (88.4–270, ±46.52, 47), SatDWE 90.3 (52.2–130, ±18.92, 47), SatDWM 99.2 (61–140, ±21.3, 47), SatDLAM 57.7 (21.9–139, ±19.73, 47), SatDLPM 122.7 (46.1–202, ±33.37, 47), SatDL/SatDWE 2 (1.4–3, ±0.36, 47), SatDL/SatDWM 1.8 (1.3–2.5, ±0.28, 47), SatDL/SatPDSW 2.2 (1.7–3, ±0.29, 47), SatDLAM/SatDL 0.3 (0.2–0.6, ±0.06, 47), SatDLAM/SatDLPM 0.5 (0.3–1.2, ±0.17, 47), SatDWM/SatDWE 1.1 (1–1.2, ±0.05, 47), SatDWM/SatPDSW 1.2 (1–1.4, ±0.09, 47). Nucleus roughly orbicular with a single, eccentrically placed orbicular karyosome: SatNL 32.5 (18.4–54.8, ±9.13, 47), SatNW 28.2 (17.5–46.3, ±8.12, 47), SatNDS 77.8 (16.2–184, ±38.2, 47), SatKL 15.3 (8.5–22.2, ±3.39, 47), SatKW 13 (6.4–20.2, ±3.1, 47). Indices: SatTL/SatPL 6 (3.1–10.3, ±1.47, 47), SatDL/SatPL 5.2 (2.6–9, ±1.37, 47), SatDWM/SatPWM 1.2 (1–1.4, ±0.1, 47), SatTL/SatDL 1.2 (1.1–1.2, ±0.03, 47).

Gametocysts: Opaque, irregularly orbicular in outline. Six gametocysts were collected and stored under moist conditions, dehiscing by simple rupture within 96 hr.

Oocysts: Strongly resembling the eggs of trichurid nematodes in general form; hesperidiform in outline, comprising a fusiform oocyst with shallowly ovoid terminal knobs or caps, with 2 large, smooth, orbicular oocyst residua and multiple minor, smooth, orbicular residua generally confined to a central, narrowly fusiform to fusiform residuum vacuole: OL 11.5 (11.1–11.8, ±0.19, 30), OW 4.8 (4.6–5.1, ±0.13, 30), OTW 1.1 (0.8–1.4, ±0.13, 30), RVL 4.5 (3.2–6.1, ±0.54, 30), RVW 1.9 (1.6–2.3, ±0.18, 30), R1D 1.4 (1–1.7, ±0.14, 30), R2D 1 (0.8–1.3, ±0.13, 30), TkL 0.7 (0.6–0.8, ±0.07, 30), TkW 1.3 (0.9–1.5, ±0.12, 30), OL/RVL 2.6 (1.9–3.6, ±0.32, 30), OL/Tkl 17.1 (13.7–20.7, ±1.8, 30), OL/R1D 8.4 (6.9–12, ±1.03, 30), OL/R2D 11.8 (9.1–15.2, ±1.6,

30), OW/OTW 4.5 (3.3–6.1, ±0.57, 30), OW/TkW 3.7 (3–5.4, ±0.42, 30), OW/RVW 2.6 (2.1–3, ±0.23, 30), OW/R1D 3.5 (3–4.9, ±0.39, 30), OW/R2D 5 (3.7–6.1, ±0.65, 30), OL/OW 2.4 (2.3–2.5, ±0.06, 30), RVL/RVW 2.4 (1.8–2.9, ±0.3, 30), TkL/TkW 0.5 (0.4–0.8, ±0.08, 30), R1D/R2D 1.4 (1.1–1.8, ±0.19, 30).

Taxonomic summary

Type host: Tropisternus collaris (Fabricius, 1775) (Coleoptera: Hydrophilidae), adults.

Type locality: Town Bluff Slough of B A Steinhagen Lake, in the western parcel of Martin Dies Jr. State Park, Cherokee Unit, Big Thicket National Preserve, Tyler County, Texas, U.S.A. (30° 33′06.2″N; 94°41′83.7″W).

Symbiotype: Five symbiotype specimens (authors' specimens REC06529–REC06534) are deposited in the Sam Houston State University Insect Collection (SHSUIC), Department of Biology, Sam Houston State University, Huntsville, Texas. Individual accession numbers are not assigned by SHSUIC.

Site of infection: Mature and immature associations were collected from the mesenteron posterior to the intercecal region. Gametocysts were collected from the host hindgut, rectum, and feces.

Prevalence: One hundred six of 129 specimens of *T. collaris* were infected (82%).

Specimens deposited: The type series is contained on 50 microscope slide preparations deposited in the Harold W. Manter Laboratory for Parasitology (HWML), Division of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska, U.S.A. The holotype is a mature association marked by an etched circle on slide HWML48512 (authors' slide REC06538A); the remaining specimens on HWML48512 are paratypes. The holotype slide is 1 of a series of 50 slides containing trophozoites, gamonts, and associations: HWML48513 (JJH060563A,B; JJH060565B; JJH060566B; JJH060567B; JJH 060568A; JJH060569A); HWML48514, (TJC 060670A, TJC060672B–D, TJC060673B, TJC 060674A, TJC060678C); HWML48515 (SJH 060116B; SJH060117B; SJH060118A; SJH 060119; SJH060120A,B; SJH060123A,B; SJH0 60124B; SJH060125; SJH060127; SJH060129; SJH060130); HWML48516 (REC06536A,B; REC 06539B,C; REC06541; REC06543A,B; REC 06547A,B; REC06548B, REC06571A,B; REC 06572A,B; REC06573; REC06575; REC06579;

REC06580A,B; REC06581; REC06582A,B). No specimen from the type series is retained by the authors.

Etymology: The specific epithet is based on the Rhine maiden Wellgunde, who appears in the first ("Das Rheingold") and fourth ("Götterdämmerung") operas of Richard Wagners' operatic cycle "Der Ring des Nibelungen." Wellgunde is a Rhine maiden or water nymph who loses the Rhinegold to Alberich the Nibelung dwarf in the opening act of "Das Rheingold" and fails to woo the resultant Nibelung ring from Siegfried in the first act of "Götterdämmerung."

Remarks

Trichurispora wellgundis is the type species of the genus. This is the first description or report of a septate gregarine infecting an adult hydrophilid beetle from the New World.

Trichurispora fusipara n. comb.
R. E. Clopton and T. J. Cook

Species inquirenda
(=*Gregarina fusipara* Baudoin and Maillard, 1972)

Description

Tropozoites: Unknown.

Association: Fusiform, precocious; PrDWM posteriad; SatDWM anteriad; PrTL 100–200; SatTL 100–200. Epimerite unknown.

Gametocysts: Unknown.

Oocyst: Unknown.

Taxonomic summary

Type host: Hydrochara caraboides (Linnaeus, 1758) (Coleoptera: Hydrophilidae), adults.

Type locality: Parc naturel régional de Brière; Nantes, Loire-Atlantique, France.

Symbiotype: None.

Site of infection: Intestine.

Other records: None.

Disposition of type specimens: No specimen from the original type series is known.

Disposition of other specimens: No permanent specimen is known.

References: Baudoin and Maillard (1972).

Table 1.   Gregarines reported from adult water scavenger beetles (Coleoptera: Hydrophilidae).

Gregarine	Host
Didymophyes cercyonis Cordua, 1953*,†	*Cercyon impressus*‡
	Cercyon lateralis‡
Didymophyes cryptopleuri Geus, 1969†	*Cryptopleurum minutum*‡
Didymophyes hydrobiina Foerster, 1938†,§	*Anacaena limbata*‡
	Enochrus frontalis‡
	Hydrobius fuscipes‡
Didymophyes lanceolata Cordua, 1953*,†	*Cercyon haemorrhoidalis*‡
	C. lateralis‡
	Cercyon quiquilius‡
Didymophyes macrocystis Cordua, 1953*	*C. impressus*‡
	C. lateralis‡
Didymophyes microcephala Cordua, 1953*	*C. impressus*‡
	C. lateralis‡
Didymophyes minima Geus, 1969†	*Cercyon pygmaeus*‡
Didymophyes sphaeridii Cordua, 1953*,†	*Sphaeridium bipustulatum*‡
	Sphaeridium scarabaeoides‡
Didymophyes sphaerocephala Cordua, 1953*,†	*S. bipustulatum*‡
	S. scarabaeoides‡
Enterocystis hydrophili (Foerster, 1938) Baudoin and Maillard, 1972‖,¶	*Hydrochara caraboides*‡
Trichurispora fusipara (Baudoin and Maillard, 1972) n. comb.‖	*H. caraboides*‡
Acanthospora polymorpha Léger, 1896†,#,**,††	*H. caraboides*‡‡
Ancyrophora umbelliformis Baudoin and Maillard, 1972#,§§	*H. caraboides*‡‡
Bothriopsides terpsichorella (Ellis, 1913) Foerster, 1938†,§,††,‖‖	*Hydrophilus sp.*‡‡
Cometoides capitatus (Léger, 1892) Labbé, 1899†,††,¶¶	*Hydrophilus sp.*‡‡
	H. caraboides‡
Cometoides crinitus (Léger, 1892) Labbé, 1899†	*Hydrobius sp.*‡‡
Cometoides pileatus Baudoin and Maillard, 1972#,¶¶	*Hydrophilus piceus*‡‡
Phialoides ornata (Léger, 1892) Labbé, 1899†,††	*H. piceus*‡‡
Stylocephalus brevirostris (von Kolliker, 1848) M. Watson, 1916†,¶,##,***	*Hydrophilus sp.*‡‡
	Hydrophilus aterrimus‡‡
	Hydrophilus sp.‡‡

* Data from Cordua (1953).
† Data from Geus (1969).
‡ Adults.
§ Data from Foerster (1938).
‖ Data from Ormières and Baudoin (1974).
¶ Incertae sedis.
# Data from Baudoin and Maillard (1972).
** Data from Léger (1897).
†† Data from Desportes (1963).
‡‡ Larvae.
§§ Data from Baudoin (1971).
‖‖ Data from Ellis (1913).
¶¶ Data from Léger (1892).
Data from von Kölliker (1848).
*** Data from Watson (1916).

Remarks

As reflected in the description above, the original report of *T. fusipara* contains little detailed information and provides no information regarding epimerite, gametocysts, gametocyst dehiscence, or oocysts. The taxon reported by Baudoin and Maillard (1972) is referable to *Trichurispora* based solely on host association, general form of the association, and the suggestion of an anterior acetabulum formed by the front margin of the protomerite (see Baudoin and Maillard [1972], plate IV, figure 4). No permanent specimen is known and no later reports exist. We deem *T. fusipara* species inquirenda rather than incertae sedis because new collections of the same taxon could be made with taxonomic confidence. Collections of characteristically "trichuisiform" oocysts will confirm the placement of this taxon in *Trichurispora*. New collections of trophozoite and

gamont material will stabilize the taxon and lead to redescription and the disposition of neotype and voucher material.

DISCUSSION

Clopton et al. (1992) coined the term "host stadium specificity" to refer to the phenomenon in which gregarines utilize only adult or only larval forms of a single host species. With the use of the developmental requirements of the gregarines parasitizing *Tenebrio molitor* as a model system, Clopton and Janovy (1993) demonstrated that the overlap of infective oocysts and hosts in the environment can be a function of host niche preference and the environmental conditions required for gametocyst development. The gregarines of hydrophilid beetles appear to reflect these phenomena at larger ecological and evolutionary scales.

Known gregarine species infecting hydrophilid hosts are presented in Table 1, with an indication of what host life-cycle stage is utilized (larval or adult beetles). Of the 13 gregarine species reported from adult water scavenger beetles, 11 are members of *Didymophyes* Stein, 1848, a group primarily associated with terrestrial beetles. All didymophyids not infecting adult hydrophilid beetles are known only from dung-eating scarab beetles (Coleoptera: Scarabaeidae) (Cordua, 1953; Ormières and Baudoin, 1974; Levine, 1988; Clopton, 2002). Coprophagy appears to be instrumental in the distribution of the genus through hydrophilids and scarabs: of those didymophyids associated with hydrophilid hosts, only 1, *Didymophyes hydrobiina* Foerster, 1938, is associated with a hydrophilid that is not a dung specialist (Hanski and Koskela, 1977; Horgan and Berrow, 2004; Sánchez Piñero and Avila, 2004). Of the remaining 2 species known from adult hydrophilid beetles, *Enterocystis hydrophili* (Foerster, 1938) Baudoin and Maillard, 1972 is not a septate gregarine and the other is reported here for the first time.

Enterocystis hydrophili has been repeatedly observed in adult hydrophilid beetles and was commonly observed in the hosts collected for this study. Despite successive reports, *E. hydrophili* remains enigmatic: The species clearly undergoes schizogony in host tissues and is thus not a septate gregarine, but the exact placement of the taxon is unclear. Until more thorough developmental studies are completed, the taxon should be considered incertae sedis and of uncertain familial placement.

Trichurispora is only the second gregarine genus reported from adult hydrophilids: All other known species utilizing adult hydrophilids are members of the genus *Didymophyes*. Evolutionarily, the emerging pattern is consistent with the initial colonization of hydrophilids by a didymophyid lineage after significant diversification within the Scarabaeidae, probably as a result of the common coprophagous habits of the adult beetles. Diversification of didymophyids within adult Hydrophilidae has progressed sufficiently to include noncopraphagous forms that share riparian or neustic habitat. Such habitats provide a nonaquatic developmental habitat for gregarine gametocysts that might be similar to that found in dung-pats. *Trichurispora* is placed within the Hirmocystidae and is a didymophyid confamilial, probably representing a separate hydrophilid colonization event. Nonetheless, members of both *Didymophyes* and *Trichurispora* are clearly part of the larger, primarily terrestrial gregarinicine lineage.

In contrast, 8 gregarine species representing at least 5 distinct genera utilize hydrophilid larvae as hosts (Table 1). *Stylocephalus brevirostris* (von Kolliker, 1848) M. Watson, 1916 is reported in Table 1 but is an enigmatic taxon not collected since its original description. Clearly not a member of *Stylocephalus* but too poorly known to be placed correctly; it should be considered incertae sedis. The remaining 7 gregarine taxa all are members of the primarily aquatic actinocephalinine lineage. The broad representation of actinocephalinine genera infecting larval hydrophilid beetles suggests that the host group has been subject to multiple colonization and diversification events.

Although gregarines are reported from both adult and larval hydrophilid beetles, no species and indeed no genus is reported from both adult and larval hosts. Holometabolic insects tend to utilize difference energy sources and microhabitats as larvae and adults, providing little ecological overlap to facilitate dual-stadium transmission by a single parasite species. Beyond the simple lack in microhabitat overlap among larval and adult hosts, differences in host microhabitat provide differential developmental habitats for the exogenous stages of their gregarine parasites. Gregarine gametocysts are enclosed within a "gametocyst envelope" of phylogenetically variable form and function. Initially appearing as a gelatinous or hyaline sheath, the gametocyst envelope is lost in the Gregarinicae but remains persistent in the Stenophoricae. Interestingly, species within Gregarinicae possess gametocysts that appear to regulate water content of the gametocyst environmentally, whereas those within Stenophoricae do not. Among the stenophorids, the gametocyst envelope

dries to form a papery outer cyst wall that prevents gametocyst desiccation in terrestrial forms but remains a gelatinous envelope in aquatic forms, apparently isolating the gametocyst and protecting it from bacterial and fungal colonization. Hydrophilid larvae are wholly aquatic in habit but hydrophilid adults readily utilize both riparian and aquatic habitats. The actinocephalinine gregarines infecting hydrophilid larvae display aquatic gametocyst adaptations, including a persistent, gelatinous gametocyst envelope. Those infecting adult hydrophilids possess nonpersistent gametocyst envelopes, an adaptation suited to moist but not submergent riparian and terrestrial habitats. These differences reflect the common requirements and adaptations of the gregarinicine and actinocephalinine lineages to terrestrial and aquatic habitats, respectively.

In both ecological and evolutionary senses, adult and larval hydrophilids represent 2 disparate habitats linked by an ontogeny. Ultimately, the gregarines infecting larval and adult hydrophilid beetles represent disparate gregarine lineages independently capturing the same host group. The primarily aquatic Actinocephalinae are presented by 5 closely related genera in wholly aquatic beetle larvae either as a result of diversification or multiple colonization. Gregarines in hydrophilid adults appear to be the descendants of a primarily terrestrial lineage that has captured semiaquatic beetle hosts.

ACKNOWLEDGMENTS

LITERATURE CITED

Baudoin, J. 1971. Etude comparée de quelques Grégarines Acanthosporinae. Journal of Protozoology 18:654–660.

Baudoin, J., and Y. Maillard. 1972. Recherches sur les Grégarines des coléoptères Hydrophilides. Protistologica 8:53–63.

Belton, P., and H. Grundfest. 1962. Potassium activation and K spikes in muscle fibers of mealworm larva (*Tenebrio molitor*). American Journal of Physiology 203:588–594.

Chakravarty, M. M. 1960. Systematic position of some genera and classification of the suborder *Cephalina* Delage and Hérouard. Proceedings of the Zoological Society of Calcutta 12:71–81.

Clopton, R. E. 2002. Phylum Apicomplexa Levine, 1970: Order Eugregarinorida Léger, 1900. Pages 205–288 *in* J. J. Lee, G. Leedale, D. Patterson, and P. C. Bradbury, eds. Illustrated Guide to the Protozoa, 2nd ed. Society of Protozoologists, Lawrence, Kansas.

Clopton, R. E. 2004a. *Calyxocephalus karyopera* g. nov., sp. nov. (Eugregarinorida: Actinocephalidae: Actinocephalinae) from the ebony jewelwing damselfly *Calopteryx maculata* (Zygoptera: Calopterygidae) in southeast Nebraska: implications for mechanical prey–vector stabilization of exogenous gregarine development. Comparative Parasitology 71:141–153.

Clopton, R. E. 2004b. Standard nomenclature and metrics of plane shapes for use in gregarine taxonomy. Comparative Parasitology 71:130–140.

Clopton, R. E. 2006. Two new species of *Xiphocephalus* in *Eleodes tricostata* and *Eleodes fusiformis* (Coleoptera: Tenebrionidae: Eleodini) from the sandhills of western Nebraska. Journal of Parasitology 92:569–577.

Clopton, R. E., T. J. Cook, and J. L. Cook. 2004. *Naiadocystis phykoterion* n. gen., n. sp. (Apicomplexa: Eugregarinida: Hirmocystidae) from the Mexican Pygmy Grasshopper *Paratettix mexicanus* (Orthoptera: Tetrigidae) in the Texas Big Thicket with recognition of three previously described species of *Naiadocystis*. Journal of Parasitology 90:301–307.

Clopton, R. E., and J. J. Hays. 2006. Revision of the genus *Protomagalhaensia* and Description of *Protomagalhaensia wolfi* n. comb. (Apicomplexa: Eugregarinida: Hirmocystidae) and *Leidyana haasi* n. comb. (Apicomplexa: Eugregarinida: Leidyanidae) parasitizing the lobster cockroach, *Nauphoeta cinerea* (Dictyoptera: Blaberidae) Comparative Parasitology 73:137–156.

Clopton, R. E., and J. Janovy, Jr. 1993 Developmental niche structure in the gregarine assemblage parasitizing *Tenebrio molitor*. Journal of Parasitology 79:701–709.

Clopton, R. E., T. J. Percival, and J. Janovy, Jr. 1992. Host stadium specificity in the gregarine assemblage parasitizing *Tenebrio molitor*. Journal of Parasitology 78:334–337.

Cordua, C. A. 1953. Untersuchungen über die Gregarineninfektion der Dungkäfer. Archiv für Protistenkunde 98:469–506.

Desportes, I. 1963. Quelques grégarines parasites d'insectes aquatiques de France. Annales de Parasitologie Humaine et Comparée 38:341–377.

Ellis, M. M. 1913. A descriptive list of the cephaline gregarines of the New World. Transactions of the American Microscopical Society 32:259–300.

Foerster, H. 1938. Gregarinen in schlesischen Insekten. Zeitschrift für Parasitenkunde 10:157–205.

Geus, A. 1969. Sporentierchen. Sporozoa. Die Gregarinida der land- und süßwasserbewohnenden Arthropoden Mitteleuropas. Die Tierwelt Deutschlands und der angrenzenden Meeresteile nach ihren Merkmalen und nach ihrer Lebensweise 57:1–608.

Grassé, P. P. 1953. Sous—Embranchement des sporozoaires. Pages 545–921 *in* P. P. Grassé, ed. Traité de Zoologie. Anatomie, Systématique, Biologie. 1(2) Protozoaires: Rhizopodes, Actinopodes, Sporozoaires, Cnidosporides. Masson et Cie, Paris, France.

Hanski, I., and H. Koskela. 1977. Niche relationships among dung-inhabiting beetles. Oecologica 28:203–231.

Harris, J. G., and M. W. Harris. 1994. Plant identification terminology: An illustrated glossary. Spring Lake Publishing, Payson, Utah. 198 pp.

Horgan, F. G., and S. D. Berrow. 2004. Hooded crow foraging from dung pats: implications for the structure of dung beetle assemblages. Biology and Environment: Proceedings of the Royal Irish Academy 104B:119–124.

Kundu, T. K., and D. P. Haldar. 1984. Observations on *Quadruhyalodiscus gallerucidae* gen. nov., sp. nov. (Apicomplexa: Sporozoea) from a Chrysomelid Beetle, *Gallerucida bicolor*. Archiv für Protistenkunde 128:327–334.

Labbé, A. 1899. Sporozoa. Das Tierreich 5:1–51.

Laird, P. W., A. Zijderveld, K. Linders, M. A. Rudnicki, R. Jaenisch, and A. Berns. 1991. Simplified mammalian DNA isolation procedure. Nucleic Acids Research 19:4293.

Lankester, E. R. 1885. Protozoa. Pages 831–865 *in* T. S. Baynes, ed. The Encyclopedia Britannica, 9th ed., vol. 19. J. M. Stoddard Co., Ltd., Philadelphia, Pennsylvania.

Léger, L. 1892. Recherches sur les Grégarines. Tablettes Zoologique 3:1–182.

Léger, L. 1897. Nouvelles recherches sur les polycystidées parasites des arthropodes terrestres. Annales de la Faculte des Sciences de Marseille 6:1–58.

Levine, N. D. 1971. Uniform terminology for the protozoan subphylum Apicomplexa. Journal of Protozoology 18:352–355.

Levine, N. D. 1988. The Protozoan Phylum Apicomplexa, Vol. I. Chemical Rubber Company Press, Boca Raton, Florida. 203 pp.

Ormières, R., and J. Baudoin. 1974. Nouvelles recherches sur les *Didymophyes*, eugrégarines parasites de Scarabaeidae et d'Hydrophilidae. Protistologica 10:77–82.

Sánchez Piñero, F., and J. M. Avila. 2004. Dung-insect community composition in arid zones of south-eastern Spain. Journal of Arid Environments 56:303–327.

von Kölliker, A. 1848. Beitrage zur Kenntniss niederer Thiere. Zietschift für wissenschaftliche Zoologie 1:1–37.

Watson, M. E. 1916. Studies on gregarines: including descriptions of twenty-one new species and a synopsis of the eugregarine records from the Myriapoda, Coleoptera and Orthoptera of the world. Illinois Biological Monographs 2:1–258.