REVISON OF THE GENUS STICTOSPORA AND DESCRIPTION OF STICTOSPORA VILLANI, N. SP. (APICOMPLEXA: EUGREGARINIDA: ACTINOCEPHALIDAE) FROM LARVAE OF THE JAPANESE BEETLE, POPILLIA JAPONICA (COLEOPTERA: SCARABAEIDAE), IN MICHIGAN

Joanna Hays, Richard E. Clopton*, David L. Cappaert†, and David R. Smitley†
Department of Natural Science, Peru State College, Peru, Nebraska 68421. e-mail: rclopton@oakmail.peru.edu

ABSTRACT: Stictospora villani n. sp. is described from larvae of the Japanese beetle Popillia japonica (Coleoptera: Scarabaeidae) from southern Michigan. Stictospora villani is distinguished from known species of the genus by differences in gamont size and by structural details of the epimerite. In general, S. villani is smaller than other known species of the genus and possesses an epimerite with a margin of 20–24 symmetrical, pendulate, narrowly to very narrowly ellipsoid lamina. Previously described species are characterized by epimerites with notably fewer marginal lamina. A heretofore unrecognized protistan is diagnosed from the coelomic fat bodies and tentatively placed within the Neogregarinorida. We conclude that previous workers have mistaken this neogregarine for the gametocyst and oocyst of Stictospora, which are described in this study for the first time. The generic diagnosis of Stictospora is revised to encompass the epimeritic variation of its constituent species and to correctly reflect the morphology of the oocyst.

Worldwide only 2 genera, Stictospora and Euspora, are reported from scarabaeid larvae (Schneider, 1875; Léger, 1893, 1896; Wellmer, 1911; Watson, 1916; Foerster, 1938; Hoshide, 1952, 1959; Obata, 1953; Theodorides, 1955, 1961; Allison, 1969; Geus, 1969). No gregarine species has been previously described or identified from the Japanese beetle Popillia japonica (Coleoptera: Scarabaeidae), although unidentified gregarines have been reported from larval Japanese beetles and June beetles in North America several times (Berberet and Helms, 1969; Regniere and Brooks, 1978; Hanula and Andreadis, 1988; Poprawski and Yule, 1992; Cappaert and Smitley, 2002). During a survey of the parasitoids and pathogens of P. japonica larvae in southern Michigan, a population of gregarines was consistently recovered from established beetle populations. These populations are morphologically and ecologically consistent with members of Stictospora but represent a taxon distinct from all known species, which is formally described in this study for the first time. After a thorough taxonomic study of the new taxon, we are able to resolve existing anomalies regarding the gametocysts and oocysts of Stictospora, and thus we in this study revise the generic diagnosis to encompass the epimeritic variation of its constituent species and to correctly reflect the morphology of the oocyst.

MATERIALS AND METHODS

Popillia japonica larvae were collected from Hidden Lake Gardens (Lenawee Co., Michigan) (42°01′52″N, 84°06′39″W), on 14 May 2000 and Medalist Golf Course outside Marshall (Calhoun Co., Michigan) (42°14′33″N, 84°58′00″W), on 19 September 2000. All larvae were collected from insecticide-free areas of rough or garden. Soil cores 10 cm deep were extracted using a 10.2-cm-diameter golf course cup cutter. Larvae were identified to species in the field, and subsamples of Japanese beetle larvae were placed in 1.0-L plastic bags filled with soil for transport in a cooler to the laboratory at Michigan State University (MSU) (East Lansing, Michigan). Larvae were packed in Styrofoam coolers with frozen gel packs and sent to the laboratory at Peru State College (PSC) (Peru, Nebraska) by overnight courier. Japanese beetle larvae were held at 10 C and examined for gregarine infection within 48 hr of receipt.

Larvae were eviscerated and their alimentary canals dissected in insect muscle saline (Belton and Grundfist, 1962). Permanent parasite preparations were made using 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 (Semichon, 1924) or Harris’ hematoxylin and eosin–xylol, and mounted in Damar balsam as described by Clopton (1995, 1999, 2000b), and Clopton and Nolte (2002). Gametocysts were collected from the hindgut and rectum during postmortem examinations, triple-rinsed in insect muscle saline, and transferred with a moist 000 paintbrush to individual glass microvials (insect genitalia vials, BioQuip Products, Gardena, California). Half the vials were stored dry, retaining only the surface moisture of the gametocyst. Aqueous 2.5% potassium dichromate (K₂Cr₂O₇) (50 µL) was added to the remaining vials. Vials were sealed with white silicon stoppers and gametocysts held for maturation and dehiscence. Gametocysts were observed daily and the appearance, form, distribution, and number of sporocytes noted. Oocyst structure and dimensions were taken from preparations of oocysts in agar monolayer mounts (Clopton, 1999, 2000b).

Observations were made using an Olympus B-Max 50 compound microscope with ×20, ×40, and ×100 universal planapochromatic objectives and with either phase contrast condensers or differential interference contrast prisms. Digital photographs were taken using an Olympus DP-11 digital camera through the aforementioned microscope. Measurements were taken from the digitized images of preserved specimens using Image-Pro Express® + 4.0 image analysis software (Media Cybernetics, L.P., Silver Spring, Maryland). Drawings were made using digitized images of live and fixed specimens. Photographic plates were processed and assembled using Adobe® Photoshop® 5.5 and Adobe® Photoshop® 7.0.1 software (Adobe Systems Inc., San Jose, California).

An extended morphometric character set for Stictospora spp. is delineated in Figures 1–3. The morphometric set is consistent with those proposed by Clopton (1999), Kula and Clopton (1999), and Clopton and Nolte (2002) but includes additional metrics particular to the genus under study. The structure of the mature trophozoite, particularly the epimerite, is diagnostic among Actinopanephidiae; thus, trophozoite morphometrics are presented in addition to gamontic morphometrics. As suggested by Filipponi (1949) and implemented by Clopton (1999), the holdfast of the taxon described in this study is considered a compound structure composed of a terminal epimerite or holdfast proper and an intercalating diamerite. Measurements are presented (µm) as mean values followed by range values, standard deviations, and sample sizes in parentheses. Terminology for parasite ontogenetic stages and anatomy largely follows that proposed by Levine (1971). Terminology for shapes of planes and solids is consistent with that suggested by Clopton (2004). Additional terminology is derived from J. G. Harris and M. W. Harris (1994).
**STICTOSPORA VILLANI, N. SP. FROM JAPANESE BEETLE LARVAE**

**FIGURES 1–3.** Diagrammatic delineation of the extended gregarine morphometric character set for *Stictospora*. 1. Oocyst. 2. Trophozoite. 3. Gamont. DEW, deutomerite equatorial width; DiL, diamerite length; DiW, diamerite width; DL, deutomerite length; DWM, deutomerite maximum width; DWMAL, deutomerite distance from anterior end to axis of maximum width; EL, overall epimerite length; HL, holdfast length; HW, holdfast width; ML, length extending from anterior end of epimerite to posterior end of deutomerite; NL, nucleus length; NSD, distance from septum to anterior end of nucleus; NW, nucleus width; OL, total oocyst length; OLI, oocyst interior length; OW, oocyst width within lateral sulcal margins; OWM, oocyst maximum width; PDSW, protomerite–deutomerite septum width; PL, protomerite length; PTW, protomerite width of truncation; PTWAL, protomerite distance from anterior end to axis of truncation; PWM, protomerite maximum width; PWMAL, protomerite distance from anterior end to axis of maximum width; TL, distance from anterior end of protomerite to posterior end of deutomerite.

**DESCRIPTION**

*Stictospora* Leâger, 1893

Revised diagnosis: Eugregarinorida Leâger, 1892, sensu Clopton (2000a); Septatorina Lankester, 1885, sensu Clopton (2000a) (= Solitaricæa Chakravarty, 1960; Actinocephalidæ Leâger, 1892, sensu Clopton (2000a); Acanthosporinae Leâger, 1892, sensu Clopton (2000a); with characters of *Stictospora* Leâger, 1893 as revised, epimerite compound with terminal epimerite holdfast proper and short intercalating diamerite; holdfast very broadly obovoid, with a margin of sympetalous, pendulate, narrowly to very narrowly elliptoid lamina, variable in number, epimerite absent in mature gamonts; association late, laterofrontal, biassociative; gametocysts spherical, hyaline coat present; sporoducts absent; oocysts irregularly dolioform with distinct lateral sulcate margins and slight axial keel; released en masse by simple rupture of gametocyst.

**Taxonomic summary**

*Type species: Stictospora provincialis* Leâger, 1893.

**Remarks**

There is some confusion surrounding the diagnosis and cardinal characters of *Stictospora*. The original diagnosis of Leâger (1893) is minimal and without illustration, the published record of a presentation before the Academy of Sciences in Paris, France. Leâger (1896) published a more complete, illustrated, and formal description of *Stictospora* 3 yr later, highlighting epimeritic and oocyst morphologies as the cardinal characters of the genus. However, the epimeritic diagnosis of *Stictospora* is cast too narrowly to encompass the variability of species now understood to comprise the genus, and the original oocyst description apparently refers to the cyst of an unrelated protistan. The genus has been revised to fully encompass its constituent species and to correctly reflect the morphology of the oocyst.

The general form of the epimerite is unique to *Stictospora* and stable across all 6 species of the genus, differing from the generic diagnosis of Leâger (1896) only in the size and number of lateromarginal lamina (Léger, 1893, 1896; Watson, 1916; Hoshide, 1952, 1959; Obata, 1953; Théodorides, 1955, 1961; Allison, 1969; Geus, 1969). Before this revision, the generic diagnosis remained largely unchanged from its monotypic erection by Leâger (1893) and reflected largely the particular structure of *Stictospora provincialis* Léger, 1893, the type species (Léger, 1896). Although the original diagnosis fixed the number of lateromarginal epimeritic lamina at 9–12, the genus clearly includes species with 20 or more lateromarginal epimeritic lamina. This discrepancy represents congeneric but interspecific variation, and the generic diagnosis of *Stictospora* is revised to encompass known variability within the taxon.

In his later formal diagnosis of *Stictospora*, Léger (1896) describes the gametocysts as spherical and dehiscing by simple rupture of the unusual reticulated outer tegument. He points out the difficulty in obtaining gametocysts, found not in the feces as expected but obtained from the tissues surrounding the “fermentation chamber” (blind rectal pouch). Although most gregarine gametocysts mature and dehisce within 10 days, Léger (1896) reports maturation after about 20 days. He describes the oocysts as biconical and axially concave in form, with a regularly punctate cyst wall in which the punctures appear to correspond to papilliform endosporic extensions (Figs. 4, 5). Among the gregarines, such oocyst ornamentation is unique to Léger’s (1896) description of *Stictospora*. His accompanying description of the type species includes a lengthy discussion of the abnormalities of the gametocyst and oocyst, not least of
which are the obvious structural and developmental problems created by the production of an 800-μm gametocyst by the fusion of 2 gamonts each exceeding 1,000 μm in length. The issue remains problematic. Since its description, S. provincialis has been reported by several authors (Wellmer, 1911; Watson, 1916; Foerster, 1938; Théodoridès, 1955; Geus, 1969), but no gametocyst or oocyst has been collected since the original description.

Gametocyst and oocyst structure are either unknown or equally enigmatic for the remaining 5 known species of Stictospora. No gametocyst or oocyst has been described for Stictospora anomalae Hoshide, 1952, Stictospora kabutomusi Hoshide, 1952, or Stictospora kurdistana Théodoridès, 1961. The gametocysts and oocysts described for Stictospora coelo- cystis Obata, 1955 and Stictospora costelytrae Allison, 1969 are consistent with those described by Léger (1896) but present similar structural and developmental anomalies. Obata (1953) reports biconical but uncurved oocysts for S. coelo- cystis dehiscent from gametocysts that are not shed in the feces, but are retained in the coelom, particularly associated with the fat bodies. He does not address the inconsistency posed by the transformation of a pair of 1,200 μm mature gamonts in the intestinal lumen into a 950-μm gametocyst associated with the coelomic fat bodies. Allison (1969) collected gametocysts of S. costelytrae from the tissues of the midgut and rectum of Cos- telytra zealandica and observed maturation and dehiscence over a series of relative humidities ranging from 60 to 100%. Of 100 gametocysts incubated for 8 mo, 5 gametocysts collected from the rectum matured after ca. 20 days of incubation in a moist chamber (Fig. 6). Similar gametocysts and oocysts were collected at PSC only by expanding the dissection to include the fat bodies. We have also collected similar gametocysts and oocysts from the fat bodies and hemocoels of scarabaeid larvae in Nebraska and Oklahoma. Whereas the gametocysts and oocysts collected from isolated P. japonica intestines at PSC are typicallygregarine in form and development, those collected at MSU and from the fat bodies and hemocoel at PSC are consistent with the anomalous reports of gametocysts and oocysts previously reported for Stictospora. On critical examination of the fat body oocysts (Fig. 6), we conclude that they are not gregarine oocysts but rather represent a heretofore unrecognized apicomplexan parasite of scarab larvae. The morphology, development, and fat body association of this protistan are consistent with a neogregarine of the Lipotrophidae Grasse, 1953, probably an undescribed species of either Lipocystis Grell, 1938 or Fari- nocystis Weiser, 1953, 2 very poorly known monotypic genera of Neogregarinorida Grasse, 1953 (Perkins, 2000). On the basis of our recognition of this neogregarine from the fat bodies of scarabaeid larvae, we conclude that previous descriptions of gametocysts and oocysts of Stictospora are consistent with neo-
gregurine and not eugregarine life cycle stages. The generic
diagnosis of *Stictospora* is revised to replace the existing ref-
terences to neogregarine forms with newly discovered eugre-
garine gametocyst and oocyst morphologies.

**Stictospora villani** Hays and Clopton, n. sp.
(Figs. 1–3, 7–17)

**Generic diagnosis:** Eugregarinororida Léger, 1892, sensu Clopton (2000a); Septatorina Lankester, 1885, sensu Clopton (2000a); Stenophorinae Levine, 1984, sensu Clopton (2000a) (= Solitaricracea Chakravarty, 1960); Actinocephalidae Léger, 1892, sensu Clopton (2000a); Acanthosporinae Le Áger, 1892, sensu Clopton (2000a); with characters of *Stictospora* Léger, 1892, sensu Clopton (2000a); Stenophoricae Levine, 1984, sensu Clopton (2000a); Acanthosporinae Le Áger, 1892, sensu Clopton (2000a); with characters of *Stictospora* Léger, 1892, sensu Clopton (2000a); Stenophoricae Levine, 1984, sensu Clopton (2000a); Stictospora villani Hays and Clopton, n. sp. (Figs. 3, 12–14); Association late, immediately be-
fore syzygy. Data reported from mature solitary gamonts. Pro-
tomerite broadly ovoid with acuminate anterior margin, PL 27.58–104.19 (59.49, ±27.78, 24), PWM 18.13–113.66 (56.52, ±35.96, 24), PL/PWM 0.78–2.92 (1.22, ±0.45, 24), PTW 13.03–89.46 (43.35, ±26.73, 24), PTWAL 7.51–31.16 (15.42, ±6.72, 24), PWMAL 14.27–60.89 (34.83, ±15.17, 24), and TL/ML 0.85–0.94 (0.89, ±0.02, 24).

**Gamonts (Figs. 3, 12–14):** Association late, immediately be-
fore syzygy. Data reported from mature solitary gamonts. Pro-
tomerite broadly ovoid with acuminate anterior margin, PL 27.58–104.19 (59.49, ±27.78, 24), PWM 18.13–113.66 (56.52, ±35.96, 24), PL/PWM 0.78–2.92 (1.22, ±0.45, 24), PTW 13.03–89.46 (43.35, ±26.73, 24), PTWAL 7.51–31.16 (15.42, ±6.72, 24), PWMAL 14.27–60.89 (34.83, ±15.17, 24), and TL/ML 0.85–0.94 (0.89, ±0.02, 24).

**Taxonomic summary**

**Host:** *Popillia japonica* (Insecta: Coleoptera: Scarabaeidae),
Japanese beetle, larvae.

**Host records:** *Popillia japonica*; larvae.

**Locality:** Hidden Lake Gardens, Lenawee Co., Michigan (42°01′52″N, 84°06′39″W); Medalist Golf Course outside Marshall, Calhoun Co., Michigan (Type Locality) (42°14′33″N, 84°58′00″W).

**Infection site:** Intestine.

**Prevalence:** Hidden Lake Gardens, 45.5% (20 of 44 larvae examined postmortem). Medalist Golf Course, 83% (15 of 18 larvae 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 slide HWML45670 (author’s slide REC00055e) is a hapantotype containing multiple trophozoites and gamonts. The paratype series includes 88 slides containing trophozoites, gamonts, and associations: Hidden Lake Gardens, Lenawee Co., Michigan (42°01’52”N, 84°06’39”W) HWML45650 (REC00006a–d), HWML45651 (REC00007a–d), HWML45652 (REC00008a–e), HWML45653 (REC00009a–c), HWML45654 (REC00011a–c), HWML45655 (REC00012a–g), HWML45656 (REC00013a–c), HWML45657 (REC00016a–f), HWML45658 (REC00019), HWML45659 (REC00020a–d), HWML45660 (REC00025a–d), HWML45661 (REC00036a–c), HWML45662 (REC00037a–e), HWML45663 (REC00041a–e), HWML45664 (REC00042a–b), HWML45665 (REC00043a–d); Medalist Golf Course outside Marshall, Calhoun Co., Michigan (Type Locality) (42°14’33’’N, 84°58’00’’W) HWML45666 (REC00052a–c), HWML45667 (REC00053a–h), HWML45668 (REC00054a–h), HWML45669 (REC00055a–d, f, g). No paratype specimen is retained by the authors.

Etymology: The specific epithet is an honorific marking the contributions of the late Michael G. Villani to the study of turf grass insects, particularly the parasites and pathogens of scarabaeid larvae.

Remarks

The species described in this study is diagnosed as a member of *Stictospora* on the basis of details of epimerite structure and general gamont shape. *Stictospora villani* is distinguished from other known species of *Stictospora* by differences in gamont size and by structural details of the epimerite. The total length of *S. villani* gamonts (392.4) is far shorter than that reported for other species, which are in some cases 6 times longer (Table I). The gamonts of *S. kurdistana* are most similar in overall gamont size, but these taxa differ dramatically in the number of epimeritic lamina. The epimerite of *S. villani* has 20–24 marginal epimeritic lamina, whereas *S. kurdistana* has only 5. Only *S. coelocystis* possess marginal lamina in numbers (20) approaching *S. villani*, but these taxa differ substantially in size and proportion; *S. coelocystis* is relatively stouter than *S. villani*. Other described species of *Stictospora* possess significantly few marginal epimeritic lamina (Table I).

### Table I. Total length (range in μm with embedded mean), ratio of protomerite length:deutomerite length (LP/LD), and number of marginal epimeritic lamina for 7 species of *Stictospora*.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Total length</th>
<th>LP/LD</th>
<th>Lamina</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Stictospora villani</em></td>
<td>144.7–392.4–691.6</td>
<td>0.19</td>
<td>20–24</td>
</tr>
<tr>
<td><em>Stictospora cotyletrae</em></td>
<td>263.0–1,035.0–1,700.0</td>
<td>0.13</td>
<td>9</td>
</tr>
<tr>
<td><em>Stictospora coelocystis</em></td>
<td>478.0–845.0–1,175.0</td>
<td>0.87</td>
<td>20</td>
</tr>
<tr>
<td><em>Stictospora kurdistana</em></td>
<td>500.0–550.0–600.0</td>
<td>0.12</td>
<td>5</td>
</tr>
<tr>
<td><em>Stictospora provincialis</em></td>
<td>1,075.0–1,385.0–1,688.0</td>
<td>0.19</td>
<td>10</td>
</tr>
<tr>
<td><em>Stictospora anomalae</em></td>
<td>940.0–1,168.0–1,430.0</td>
<td>0.08</td>
<td>10–12</td>
</tr>
<tr>
<td><em>Stictospora kabutomusii</em></td>
<td>892.0–1,153.0–1,560</td>
<td>0.12</td>
<td>12–14</td>
</tr>
</tbody>
</table>

* Data from Allison (1969).
† Data from Obata (1953).
‡ Data from Théodorides (1955).
§ Data from Geus (1969).
|| Data from Hoshide (1952).

### DISCUSSION

Although the role of gregarine infection in the colonization and population dynamics of *P. japonica* is as yet unclear, the parasite is widespread. In related studies, we have recovered *S. villani* from larval populations of *P. japonica* in 6 states across the midwest and northeastern United States, i.e., Michigan, Ohio, New York, Connecticut, and Rhode Island. Three previous studies document patterns of gregarine prevalence (and more specifically *S. villani* prevalence) in larval populations of *P. japonica*. Regniere and Brooks (1978) reported 2.3–100% prevalence from 20 sites in North Carolina, Hanula and Andreadis (1988) found 55–96% prevalence in populations in Connecticut, and Cappaert and Smitley (2002) reported an average prevalence of 33% across 35 sites in Michigan. The photgraphic plates of Regniere and Brooks (1978) and Hanula and Andreadis (1988) are morphologically consistent with *S. villani*, and we confirmed *S. villani* as the primary gregarine in the Michigan study by detailed morphological examination of subsamples of the gregarines collected. Although the dynamics of population establishment and stability are known for no gregarine species, Cappaert and Smitley (2002) reported significantly different *S. villani* prevalence rates among established and recent populations of *P. japonica*. Among sample sites with *S. villani* infections, established *P. japonica* populations (established >20 yr ago) exhibited average *S. villani* prevalence rates of 53.6%, whereas the average prevalence in *P. japonica* populations established less than 10 yr ago was only 38.8%. These observations suggest that although *P. japonica* spreads to new localities as the Japanese beetle colonizes new territory, the prevalence of gregarine infection increases with long-term habitation.

### ACKNOWLEDGMENTS

This material is based in part on the work supported by the National Science Foundation under Grant 9705179 to R.E.C. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. We (J.H. and R.E.C.) thank the Offices of the President and Vice-President for Academic Affairs, Peru State College, for their generous provision of facilities and resources to support this work.