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Leidyana canadensis N. Sp. (Apicomplexa: Eugregarinida) from Larval Eastern Hemlock Looper, *Lambdina fiscellaria fiscellaria* (Lepidoptera: Geometridae)

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ABSTRACT. *Leidyana canadensis* n. sp. (Apicomplexa: Eugregarinida) is described from larvae of the eastern hemlock looper, *Lambdina fiscellaria* (Lepidoptera: Geometridae) collected near St. Stephen, Charlotte County, New Brunswick, Canada. Gamonts solitary, located between host ventricular peritrophic membrane and ventricular epithelium. Protomerite very broadly ellipsoid with transverse posterior margin; length 43.7 μm, width 42.7 μm; protomerite-deutomerite septum strongly constricted. Deutomerite narrowly obovoid with anterior transverse margin; length 186.6 μm, width 58.7 μm; total length 227.1 μm. Nucleus spherical to broadly ellipsoid; length 26.3 μm, width 20.2 μm; placement abaxial in the posterior ⅔ of the deutomerite. Nucleus often obscured in late gamonts. Association late, caudofrontal, ephemeral and leading directly to syzygy. Gametocysts roughly spherical; diameter 216.7 μm; hyaline coat increasing diameter to 359.1 μm. Gametocysts mature and dehisce through six short spore tubes within 48 hours. Oocysts axially symmetric, dolioform in dorsal aspect, compressed in the plane perpendicular to the major axis, very uniform in size and shape; length along major axis 5.2 μm, terminal width 1.8 μm, medial width 3.8 μm; extruded in chains.

Supplementary key words. Gregarine, gregarinidae, parasite, protista, protozoa, septatorina, sporozoea.

THE genus *Leidyana* Watson (1915) was erected to comprise those gregarine species possessing a simple, globular epimerite, "solitary sporonts," gametocyst dehiscence by spore ducts, and dolioform oocysts [36]. Solitary sporonts define the superfamily Stenophoricae, whose members delay reproductive association until the onset of syzygy. In contrast, members of the superfamily Gregarinicae form reproductive associations early and maintain these associations throughout their growth and development. The timing of reproductive association correlates strongly with mechanisms of gametocyst dehiscence. Gametocyst dehiscence by simple rupture is almost universal among the Stenophoricae while gametocyst dehiscence through spore ducts is a feature of the Gregarinicae observed in no stenophorid taxon except *Leidyana.* Thus *Leidyana* possess strongly correlated character suites of both Stenophoricae and Gregarinicae. The systematic arrangements of Chakravarty [7] and Levine [27] utilize the genus *Leidyana* as a linchpin for their superfamily hypothesis based on association timing. This hypothesis reconciles reproductive association with a century-old family level arrangement based on gametocyst dehiscence [37, 38].

Leidyana comprises 28 species and is cosmopolitan in distribution. All known *Leidyana* species are parasites of insects and are reported from lepidopteran larvae in California [29], England [22], France [11, 15, 31], India [16], Japan [19], and Venezuela [14]; orthopterans in Illionois, New York, Pennsylvania [36], France [10, 12, 15], India [17, 18, 30, 34], and Japan [20, 21]; coleopterans in Germany [6, 15] and India [32, 33]; trichopteran larvae in France [2, 3] and Germany [15]; a hymenopteran in India [5]; and one blattodid roach species in Texas [8]. The diagnostic characters of the genus, (a simple, globular epimerite; solitary sporonts; gametocyst dehiscence by spore ducts; and dolioform oocysts), are observed in four distinct ontogenetic stages, (trophozoites, gamonts, mature gametocysts, and oocysts, respectively) and the complete ontogeny of any putative *Leidyana* species must be observed in order to confidently ascribe that species to the genus [8]. These ontogenetic details are known for only 18 of the 28 species currently constituting *Leidyana,* thus taxonomic studies of the group simultaneously refine our understanding of *Leidyana* and test our current systematic hypothesis for the superfamilies of Septatina [9].

During a study of the population dynamics of the eastern hemlock looper, *Lambdina fiscellaria fiscellaria* (Guenée), (Lepidoptera: Geometridae) CJL discovered a high incidence of gregarine parasitism in larval looper populations. A complete study of the parasite's life cycle was conducted and the diagnostic characters of the genus *Leidyana* were observed. The gregarine populations recovered from *L. fiscellaria* are taxonomically distinct from all described species of *Leidyana.* The unique morphology of this gregarine population prompted the present proposal of a new species of eugregarine.

MATERIALS AND METHODS

Field sampling and laboratory rearing. *Lambdina fiscellaria* larvae were collected for laboratory rearing from a mixed softwood-hardwood forest site off the Mohannes Road near St. Stephen, New Brunswick, Canada. Host samples were collected between June 15 and August 17, 1993 (11 samples) and between June 10 and August 15, 1994 (16 samples). Larvae were collected by beating branches of fir trees (*Abies balsamea* (L.) Mill.) with a 1-m long stick, dislodging larvae into a 60 × 40 cm tray placed under the branch. They were transported to the laboratory in a covered seven-liter plastic tub containing fresh balsam fir foliage and individually reared in clear plastic one-

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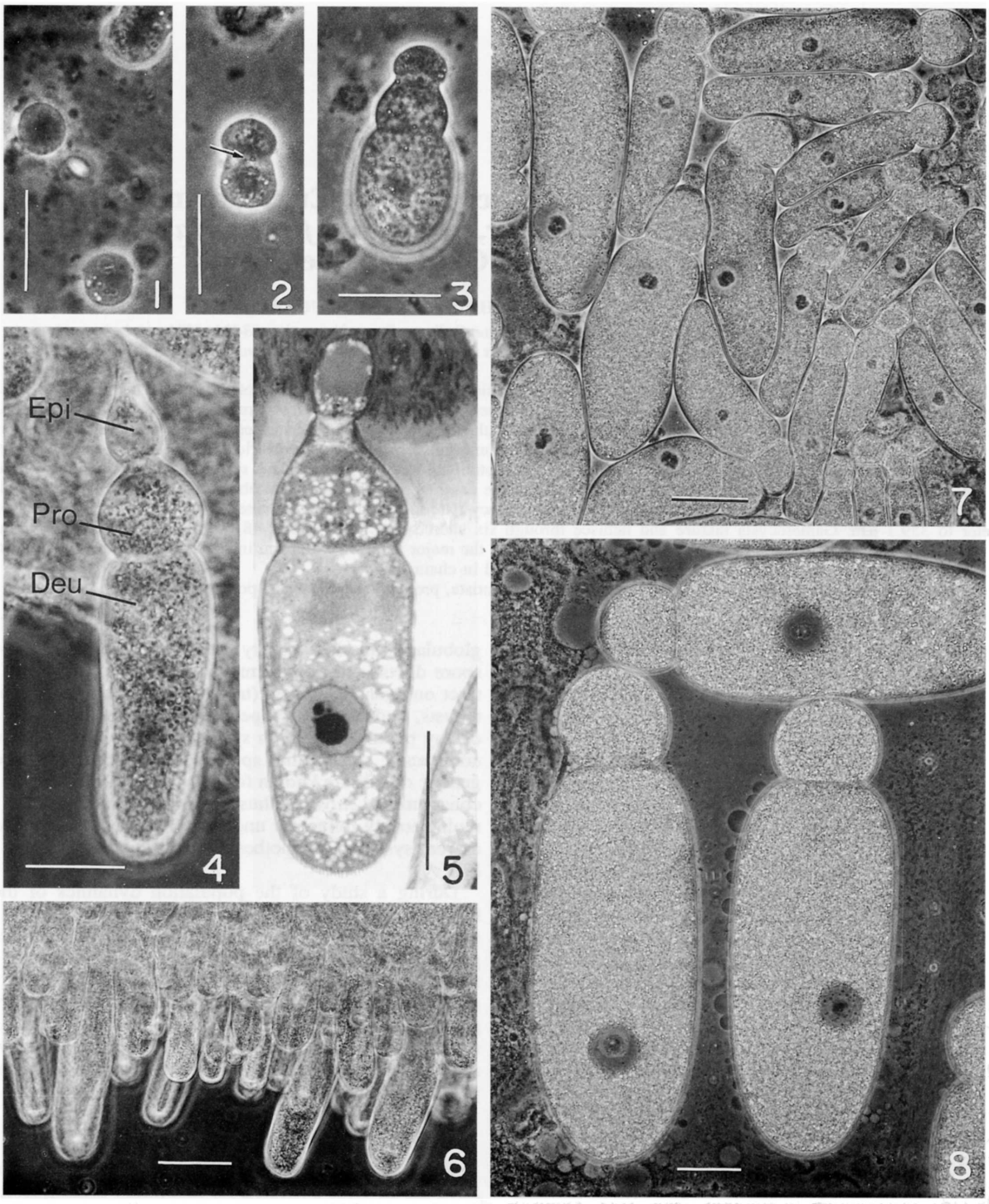
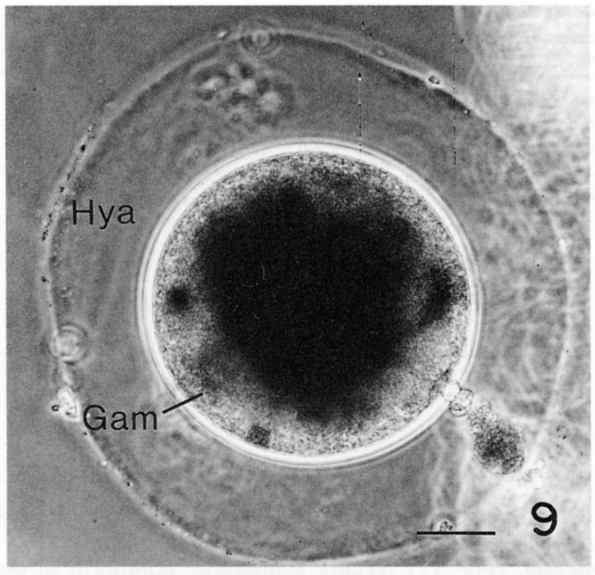
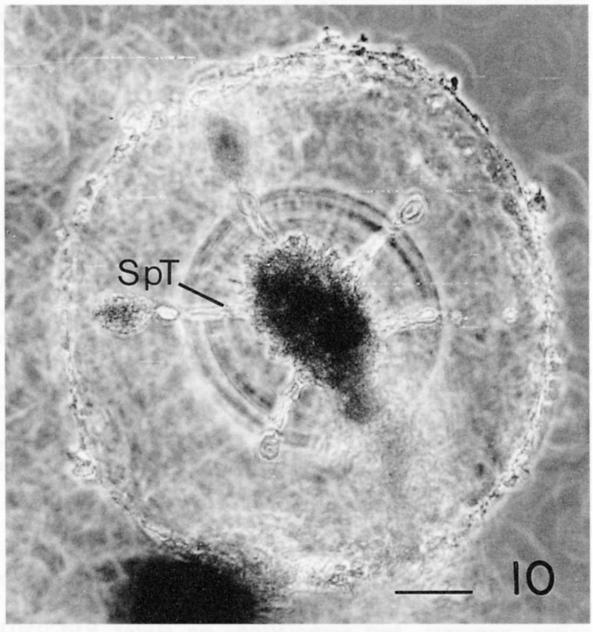


Fig. 1–8. Trophozoites and gamonts of *Leidyana canadensis* n. sp. **1.** Young trophozoite prior to formation of the protomerite-deutomerite septum. Bar = 20 μm. **2.** Protomerite-deutomerite septum (arrow) develops before epimerite in young trophozoites. Bar = 20 μm. **3.** Young trophozoite with distinct epimerite, deutomerite and protomerite. Bar = 20 μm. **4.** Trophozoite attached to host intestinal epithelium. Bar = 20 μm. **5.** Cross-section of trophozoite showing host-parasite interface. Bar = 20 μm. **6.** Solitary trophozoites attached to host. Bar = 40 μm. **7.** Solitary trophozoites and gamonts. Bar = 40 μm. **8.** Solitary gamonts. Bar = 40 μm. Deu, deutomerite; Epi, epimerite; Pro, protomerite.

ounce containers at 22° C with 16L:8D photoperiod. Larvae were checked daily and fresh, sterilized foliage was supplied as required. Fir foliage was surface sterilized by rinsing in a 2% solution of commercial bleach for 10 min, followed by a 10 min rinse in 1% bleach and two 10 min rinses in demineralized water.

Morphometric data collection. *Lambdina fiscellaria* larvae were eviscerated and their alimentary canals were dissected in





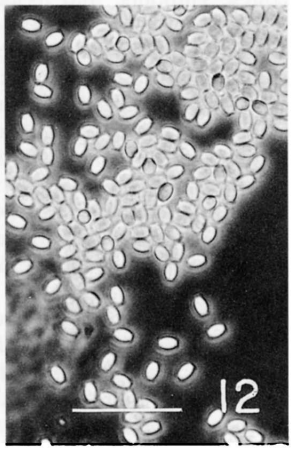


Fig. **9–12.** Gametocysts and oocysts of *Leidyana canadensis* n. sp. **9.** Developing gametocyst with outer hyaline coat. Bar = 50 μm. **10.** Mature gametocyst dehiscing through six spore tubes. Bar = 50 μm. **11.** Oocyst chains (arrows) extruded from a mature gametocyst dehiscing in looper frass. Bar = 250 μm. **12.** Oocysts. Bar = 25 μm. Gam, gametocyst; Hya, hyaline coat; SpT, spore tube.

insect muscle saline [4]. Smear preparations of host intestinal tissue and luminal contents were simultaneously fixed and stained for 2 min in Semichon's acetocarmine [35], dehydrated in ethanol, cleared in xylene, and mounted in Damar balsam [13]. Gametocysts were collected from frass and transferred into individual wells of a tissue culture plate. 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 three-dimensional structure was observed.

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 with embedded means followed by standard deviations in parentheses unless otherwise noted. Terminology for parasite ontogenetic stages follows that proposed by Levine [26]. Terminology for shapes of planes and solids is consistent with that suggested by the Systematics Association Committee for Descriptive Biological Terminology [1].

Observations and measurements were made using an Olympus EH–2 and BioScan Optimas v 4.1 image analysis software (BioScan Inc., Edmonds, WA) using input from a Javelin Ultrachip high resolution camera. Photomicrographs were taken using a Leitz Aristoplan photomicroscope.

Cytology. Intestines of infected larvae were dissected into fixative (2.5% glutaraldehyde in 0.05M Na-cacodylate buffer—0.1M sucrose pH 7.4) and quickly embedded in molten, low gelling temperature agarose (FMC Corp., Rockland, MD) to preserve the spatial integrity of the luminal contents. Embedded intestines were cut into 3–5 mm pieces, re-embedded in agarose and placed in fresh fixative for 1–3 h. After primary fixation, samples were rinsed for 15 min in each of three 0.05M Na--cacodylate solutions containing 0.1M, 0.05M and 0.00M sucrose, respectively. Specimens were secondarily fixed for 15 min in 1% osmium tetroxide in 0.05M Na-cacodylate buffer and triple-rinsed (15 min each) in 0.05M Na-cacodylate buffer. Specimens were stained with 2% aqueous uranyl acetate en bloc overnight, in the dark, at 4° C. Specimens were dehydrated in a graded acetone series and embedded in Epon-Araldite. Sections 1 μm thick were cut using a Diatome histo-knife and stained with toluidine blue for light microscopy.

RESULTS

Leidyana canadensis n. sp. (Fig. 1–12)

Diagnosis. Eugregarinida Lèger, 1892 [25], sensu strictu Levine, et al. [28]; Septatina Lankester, 1855 [24], sensu strictu Levine, et al. [28]; Stenophoricae Levine, 1984 [27], sensu Solitaroidea Chakravarty [7]; Leidyanidae Kudo, 1954 [23]; with the characters of the genus *Leidyana* Watson, 1915 [36]: association late; epimerite simple, globular, sessile; gametocysts dehiscing through sporoducts; oocysts dolioform, released in long chains.

Trophozoite. (Fig. 1–7) Very young trophozoites roughly spherical and without protomerite-deutomerite septum (Fig. 1); protomerite-deutomerite septum developing before epimerite (Fig. 2, 3). Developing trophozoites (n = 120, except as noted)

Table 1. Comparison of gamont morphometric data from four species of *Leidyana*.

	Leidyana bimaculata[a]	*Leidyana canadensis*	*Leidyana latiformis*[b]	*Leidyana suzumushi*[c]
Total length (TL)	178.8 μm	227.1 μm	240.8 μm	315.0 μm
Protomerite length (PL)	35.0 μm	43.7 μm	39.3 μm	52.0 μm
Deutomerite length (DL)	150.0 μm	186.6 μm	201.5 μm	263.0 μm
Protomerite width (PW)	51.0 μm	42.7 μm	53.8 μm	53.0 μm
Deutomerite width (DW)	62.5 μm	58.7 μm	87.3 μm	73.0 μm
PL/TL	0.20	0.20	0.16	0.17
DL/TL	0.84	0.81	0.84	0.83
PL/DL	0.23	0.31	0.20	0.20
PW/PL	1.46	1.02	1.37	1.02
DW/DL	0.42	0.33	0.43	0.28
PW/DW	0.82	0.88	0.62	0.73

[a] Data from Hooger & Amoji (1986) [18].
[b] Data from K. Hoshide (1973) [20].
[c] Data from H. Hoshide (1958) [19].

attached to host ventricular epithelium, solitary, nonassociative (Fig. 4–6). Protomerite very broadly ovoid with transverse posterior margin; length (LP) 4.3–**16.1**–29.0 (4.9 ±), width (WP) 6.6–**19.2**–36.9 (6.0 ±), WP/LP 0.76–**1.23**–2.99 (0.28 ±). Deutomerite obovoid with transverse anterior margin; length (LD) 8.6–**51.6**–114.9 (22.5 ±), width (WD) 8.5–**27.4**–66.8 (11.7 ±), WD/LD 0.33–**0.57**–1.34 (0.20 ±). Total length excluding epimerite (TL) 16.0–**70.3**–141.0 (26.1 ±). Indices: WP/WD 0.55–**0.74**–1.13 (0.13 ±), LP/LD 0.21–**0.34**–0.75 (0.11 ±), LP/TL 0.17–**0.24**–0.38 (0.05 ±), LD/TL 0.38–**0.72**–0.92 (0.10 ±). Epimerite depressed ovoid, posterior margin recurving at junction with protomerite, without a visible septum; length (LE) 2.1–**9.0**–17.90 (3.1 ±), width (WE) 2.2–**10.1**–19.6 (3.3 ±), WE:LE 0.66–**1.18**–2.22 (0.31 ±). Nucleus (n = 114) broadly ellipsoid, typically equatorial but often in medial posterior 2/3 of deutomerite; length (LK) 3.5–**11.7**–18.5 (3.5 ±), width (WK) 3.1–**11.1**–18.8 (3.3 ±), WK/LK 0.65–**0.97**–1.41 (0.14 ±). Variation in trophozoite morphology reflects both maturation and normal population variability. Trophozoites solitary (Fig. 6, 7).

Gamont. (Fig. 7, 8) Free, located between host ventricular peritrophic membrane and ventricular epithelium, solitary (n = 230, except as noted). Epimerite usually absent, shed with maturity. Protomerite very broadly ellipsoid with transverse posterior margin; LP 14.1–**43.7**–201.9 (27.4 ±), WP 14.4–**42.7**–110.7 (23.8 ±), WP/LP 0.17–**1.02**–1.68 (0.26 ±); with strong posterior constriction at protomerite-deutomerite septum. Deutomerite narrowly obovoid with anterior transverse margin; LD 42.2–**186.6**–403.9 (94.5 ±), WD 20.5–**58.7**–190.8 (42.6 ±), WD/LD 0.09–**0.33**–0.89 (0.15 ±). TL 60.1–**227.1**–463.6 (106.1 ±). Indices: WP/WD 0.52–**0.80**–2.25 (0.23 ±), LP/LD 0.14–**0.31**–4.78 (0.59 ±), LP/TL 0.13–**0.20**–0.83 (0.09 ±), LD/TL 0.17–**0.81**–0.91 (0.09 ±). Nucleus spherical to broadly ellipsoid (n = 208); LK 8.1–**21.9**–51.8 (9.6 ±), WK 8.0–**17.8**–45.9 (8.7 ±), WK/LK 0.47–**0.83**–1.32 (0.18 ±); placement abaxial in the posterior ⅔ of the deutomerite. Nucleus often obscured in late gamonts.

Association. Caudofrontal; late and ephemeral, leading directly to syzygy; 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. (Fig. 9–12) White to amber, roughly spherical; diameter 190.0–**216.7**–240.0 (17.2 ±), (n = 34); hyaline coat uniform, clear, increasing diameter to 330.0–**359.1**–390.0 (21.67 ±), (n = 34), observed on fresh gametocysts and retained under high humidity (Fig. 9–10) but rapidly drying to a surface film under low humidity (Fig. 11). Gametocysts mature and dehisce through short spore tubes (usually six) within 48 h (Fig. 10–11). Oocysts are extruded in chains (Fig. 11).

Oocysts. (Fig. 12) Axially symmetric, dolioform in dorsal aspect, compressed in the plane perpendicular to the major axis, very uniform in size and shape: length along major axis 4.6–**5.2**–6.1 (0.3 ±), terminal width 1.3–**1.8**–2.3 (0.3 ±); median width 3.0–**3.8**–4.3 (0.3 ±), (n = 82).

Type host. *Lambdina fiscellaria fiscellaria* (Guenée) (Insecta: Lepidoptera: Geometridae), Eastern Hemlock Looper; larvae.

Symbiotype. One symbiotype specimen was preserved and stored in 80% ethanol and deposited in the Division of Entomology, University of Nebraska State Museum, Lincoln, NE. The symbiotype is identified with a collection label "CANADA New Brunswick St. Stephen, June 1994 Coll. C. J. Lucarotti," and a blue voucher label "RESEARCH PROJECT Voucher Specimen."

Type locality. St. Stephen, Charlotte County, New Brunswick, Canada.

Specimens deposited. Holotype and paratype specimens were 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 39252 (author's slide REC950194) and is marked by an etched circle. The remaining trophozoites, gamonts, and associations on HWML 39252 are paratypes. Trophozoites, gamonts, and associations on slides REC950189–REC950193 and REC950195–REC950213 are paratypes retained in the personal collection of REC.

Infection site. Trophozoites and gamonts were observed in the anterior two-thirds of the ventriculus. Associations were observed in the posterior ventriculus, anterior to the ileum and the attachment of the Malpighian tubules. All endogenous lifecycle stages were observed between host ventricular peritrophic membrane and ventricular epithelium. Trophozoites were attached to the ventricular epithelium.

Etymology. The specific epithet is given to mark the discovery of this parasite within the boundaries of Canada.

Remarks. *Leidyana bimaculata* Hooger and Amoji, 1986 [18], *Leidyana suzumushi* K. Hoshide, 1973 [20], and *Leidyana latiformis* H. Hoshide, 1958 [19] resemble *L. canadensis* more closely than do other described species of *Leidyana*. Both morphometric and ecological characters differentiate *L. canadensis* from *L. latiformis, L. bimaculata,* and *L. suzumushi*. Although the morphometric ranges of all four species overlap, individual mature gamonts of *L. canadensis* are generally longer than those of *L. latiformis* and *L. bimaculata* but shorter than mature

gamonts of *L. suzumushi* (Table 1). Mature gamonts of *L. canadensis* are also relatively slender in comparison to gamonts of *L. latiformis, L. bimaculata,* or *L. suzumushi.* Individuals of *L. canadensis* possess an ovoid to elliptical protomerite that is distinctly different from the conical protomerites of *L. latiformis, L. bimaculata,* and *L. suzumushi. Leidyana canadensis* is reported from larvae of a geometrid moth, *L. fiscellaria,* while *L. bimaculata,* and *L. suzumushi* are reported from orthopterans in India and Japan, respectively [18, 20]. *Leidyana latiformis* is reported from larvae of a tineid moth in Japan [19]. Although some flexibility in host utilization has been reported for *Leidyana* species infecting orthopterans, this flexibility is apparently restricted to congeneric host species and probably does not extend across host orders [10]. Thus, in addition to geographical separation, presumed differences in host utilization serve to isolate *L. canadensis* from *L. latiformis, L. bimaculata,* and *L. suzumushi.*

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