

The Life Cycle of *Leidyana ampulla* sp. n. (Apicomplexa: Eugregarinorida: Leidyaniidae) in the Grasshopper *Ronderosia bergi* (Stål) (Orthoptera: Acrididae: Melanoplinae)

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Summary. *Leidyana ampulla*, a new species of septate eugregarine, is described from the Argentine grasshopper *Ronderosia bergi*. Prevalence was high (76 %, n = 50) in San Pedro, Misiones province in northeastern Argentina, but the parasite was not detected in the center of the country (Bagual and Buena Esperanza in San Luis province, and Pehuajó in Buenos Aires province) where *R. bergi* is also normally an abundant species. Trophozoites, which had a simple, globular epimerite and were solitary, occurred attached to the intestinal epithelium. During transition from trophozoite to gamont the epimerite was not shedded but retracted into the protomerite. Gamonts were solitary, had a characteristic bottle-like appearance, and a total length that ranged from 280 to 584 μm (mean: 526.2 ± 13.3). Syzygy was biassociative and caudofrontal, the associates resembled each other in shape but not size. Spherical gametocysts measured 104 to 360 μm (mean: 247.7 ± 49.3). Gametocyst dehiscence was by a variable number of sporoducts (up to 12). Oocysts were dolioform, measuring 5.7 ± 0.06 by 2.8 ± 0.08 μm .

Key words: Argentina, grasshopper, gregarine, *Leidyana*, parasite, Protozoa.

INTRODUCTION

The melanopline grasshopper *Ronderosia bergi* (Stål) is widely distributed in southern South America, occupying eastern North and central Argentina, Uruguay, most of Paraguay, the southeastern tip of Bolivia, and southeastern Brazil (COPR 1982, Cigliano 1997). Dam-

age to crops and forage by *R. bergi* has been reported in some areas (Ronderos 1959, COPR 1982). In spite of the common occurrence and economic importance of *R. bergi*, virtually nothing is known about the parasites and pathogens that are associated to it. In recent years, while conducting surveys in search for natural enemies of grasshoppers in Argentina, we noticed an undescribed septate eugregarine (Eugregarinorida: Septatorina) parasitizing *R. bergi*. The present study describes the new gregarine, *Leidyana ampulla* sp. n., based on life cycle observations, and provides information on its natural occurrence.

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MATERIALS AND METHODS

Older nymphs (fourth and fifth instars) and adults of *R. bergi* were collected with sweep nets in fields at the vicinity of the localities of San Pedro (northern Misiones province), Pehuajó (western Buenos Aires province), and Bagual and Buena Esperanza (southern San Luis province), areas where *R. bergi* is normally common. The samples were immediately transferred to the Center for Parasitological Studies and Vectors (CEPAVE) where the grasshoppers were either frozen at -32°C upon arrival for later examination or were maintained in groups in wire-screened cages in rearing rooms under controlled conditions (30°C , L:D = 14:10, 40 % RH) as described by Henry (1985) but without the addition of antibiotics in the diet. Grasshoppers in cages were kept for several weeks and individuals were frequently examined in order to conduct the observations. Frozen samples were employed for estimating the natural prevalence, infection intensity, and location of the gregarine within the host. All grasshoppers collected in Pehuajó ($n = 108$) and Bagual ($n = 103$) were frozen. Grasshoppers from San Pedro and Buena Esperanza were either frozen ($n = 50$ for San Pedro; $n = 100$ for Buena Esperanza) or kept in cages ($n = 44$ for San Pedro; $n = 30$ for Buena Esperanza) in two different rooms.

The grasshoppers, thawed or alive, were examined by longitudinal, ventral dissection under a stereo zoom microscope. Before dissecting living individuals, a droplet of haemolymph was obtained by pulling a leg off. Haemolymph samples were readily examined as fresh preparations under a phase-contrast microscope ($400\times$, $1000\times$). Fresh mounts of host intestinal tissue and luminal contents were prepared either with or without a small drop of one-quarter-strength Ringer's solution (Poinar and Thomas 1984), and observed and photographed under phase-contrast microscopy. Some entire alimentary canals were removed, fixed in alcoholic Bouin's fluid, embedded in paraffin, sectioned at 3-5 μm , and stained with Heidenhain's haematoxylin (Becnel 1997). Gregarine gametocysts were recovered with a capillary tube appressed to incised midgut or hindgut tissues or with a delicate brush from faeces, and transferred to Petri dishes containing moistened filter paper, where they were held at room temperature for maturation and dehiscence. Oocysts were obtained from chains dehisced from gametocysts and suspended in double distilled water when desired. Emergence of sporozoites was induced by placing oocysts in fresh mounts of host digestive tract extracts as described by Hoshida *et al.* (1994). The developmental stages, gametocysts, and oocysts from fresh mounts were measured using an ocular micrometer. Terminology for the stages of the gregarine follows Levine (1971).

For scanning electron microscopy, oocysts were fixed in 2.5% (v/v) glutaraldehyde buffered with 0.1 M cacodylate buffer (pH 7.4), dried in a critical point dryer or treated with hexamethyldisilazane (Nation 1983, Lange 1993), coated with gold-palladium, and photographed with a JEOL-JSM-T100 electron microscope.

RESULTS

The gregarine was detected in 76 % of the frozen grasshoppers from San Pedro, and was present in increasing intensity as time went by in grasshoppers kept

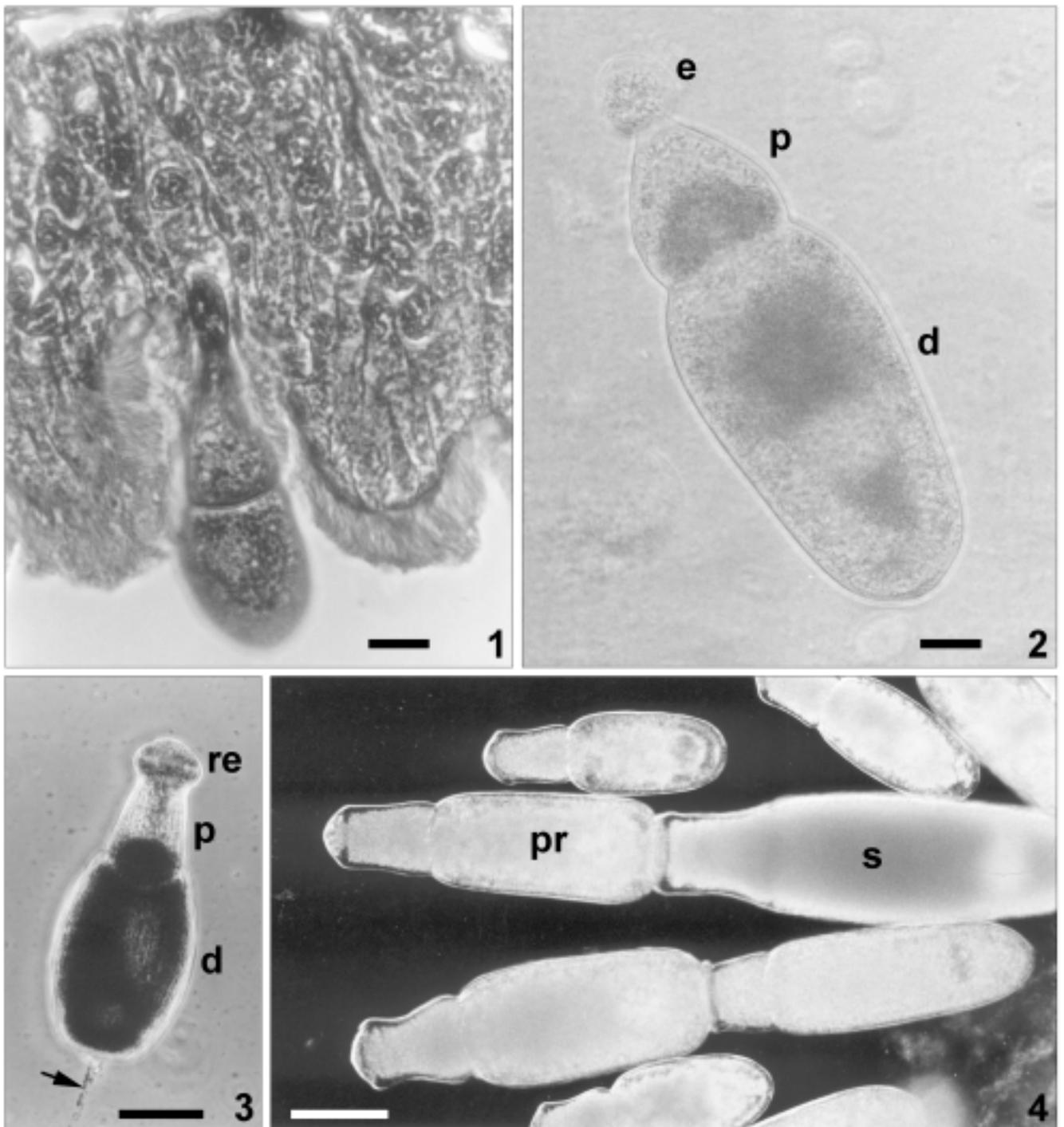
in cages from the same locality. Samples from Bagual, Buena Esperanza, and Pehuajó never revealed the presence of the parasite. Detection of the gregarine in frozen samples was by observation of some solitary gamonts in the gut and gastric caecae. Trophozoites, gamonts in association, and gametocysts were not observed in grasshoppers from frozen samples. On the contrary, the presence of the gregarine in *R. bergi* from San Pedro held in cages for prolonged periods of time was abundant, as attached trophozoites (Fig. 1) and solitary gamonts (Fig. 3) in midgut and gastric caecae, and as gametocysts (Figs 5, 6) in midgut, hindgut and faeces. The gregarine was never observed in haemolymph samples.

Very young, unsegmented or segmenting trophozoites were not seen. The earliest trophozoites observed were slender, solitary bodies that were typically divided into epimerite, protomerite, and deutomerite, and were attached to the intestinal epithelium (Fig. 1). Only once a trophozoite was seen free (i.e. unattached to the host intestinal epithelium), and its epimerite was simple and globular (Fig. 2). Its total length was 103 μm .

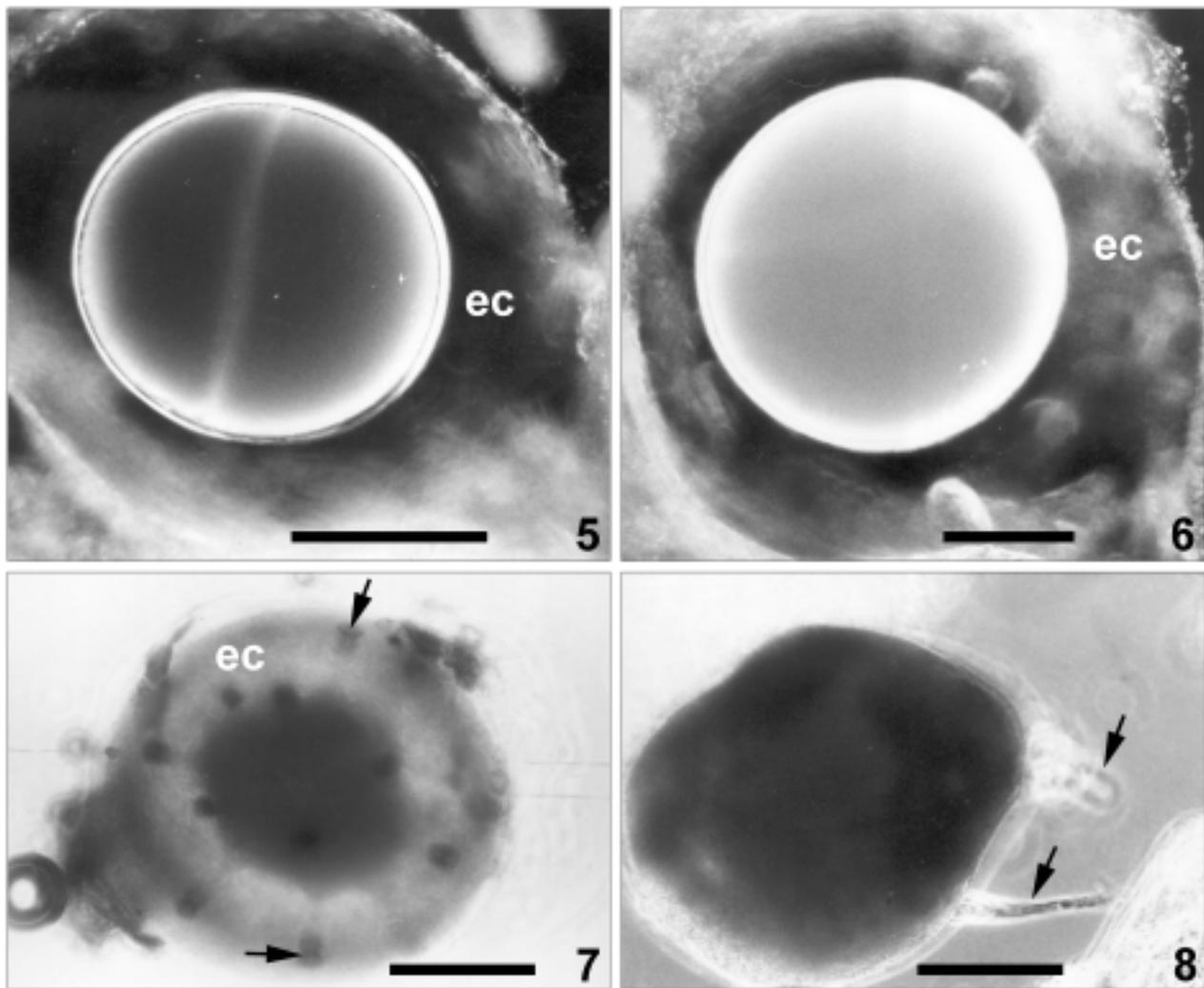
Gamonts (Figs 3, 4), the most common developmental stages observed, were normally whitish, and visible through the wall of the midgut and gastric caecae under the dissecting microscope. Gamonts ranged in length from 280 to 584 μm (Table 1) and had a slender, bottle-like appearance. They were solitary and showed progressive locomotion by gliding (Fig. 3). The epimerite of trophozoites appeared to retract into the protomerite during the transition trophozoite/gamont because a scar where the epimerite would have been eventually shed

Table 1. Range and mean (\pm SE) measurements in μm of gamonts of *Leidyana ampulla* sp. n. ($n = 30$). (TL) total length, (LP) length of protomerite, (LD) length of deutomerite, (WP) width of protomerite, (WD) width of deutomerite.

Character	Range	Mean
TL	280-584	526.2 \pm 13.3
LP	96-192	129.8 \pm 5.3
LD	180-464	396.4 \pm 12.4
WP	72-160	133.3 \pm 4.8
WD	104-296	223.1 \pm 8.5
TL:LD	1 : 1.2-1.7	1 : 1.3
WD:WP	1 : 1.3-2	1 : 1.7
TL:LP	1 : 2.4-5.1	1 : 4.2
LD:WD	1 : 1.2-2.3	1 : 1.8
WP:LP	1 : 0.6-1.4	1 : 1.1



Figs 1-4. Trophozoites and gamonts of *Leidyana ampulla* sp. n . **1** - young trophozoite attached to midgut epithelium; **2** - unattached trophozoite; **3** - solitary, bottle-like shaped gamont leaving a trail of unknown material (arrow) while gliding forward. Also note the absence of a scar in the protomerite where the epimerite was located, and the bulb shape of the anterior end of protomerite where the epimerite was retracted; **4** - solitary gamonts and gamonts in biassociative, caudofrontal syzygy. e - epimerite, d - deutomerite, p - protomerite, pr - primate, re - retracted epimerite, s - satellite. Scale bars 10 μ m (1, 2); 100 μ m (3, 4).

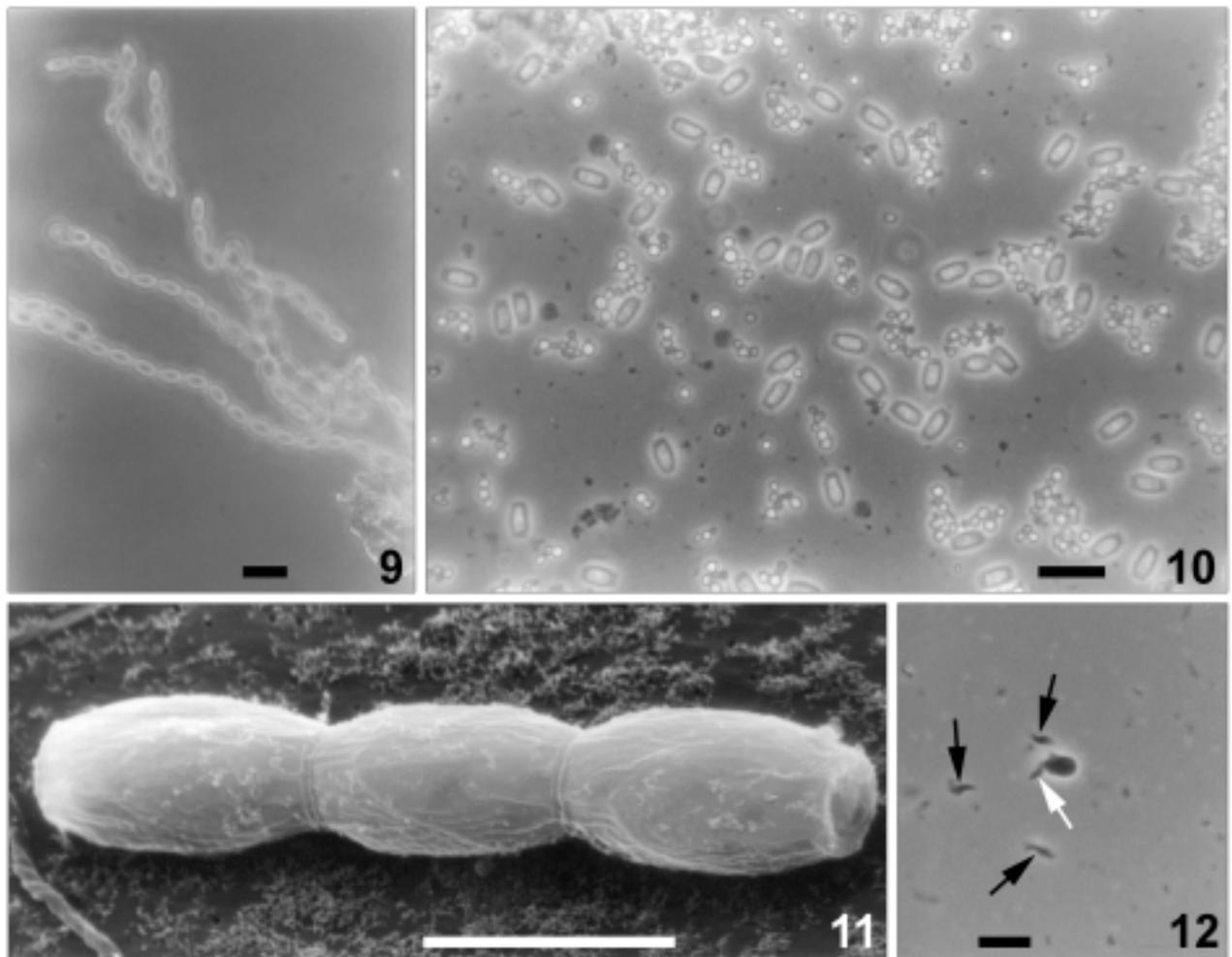


Figs 5-8. Gametocysts of *Leidyana ampulla* sp. n. **5** - young gametocyst in which members of the syzygyal association are still in separate condition; **6** - immature gametocysts; **7** - mature gametocyst showing basal discs of sporoducts (arrows); **8** - disrupted gametocyst showing two everted sporoducts (arrows). ec - ectocyte. Scale bars 100 μ m (5-8).

was never seen in protomerites of gamonts, and these also showed an evident thickening at the protomerite's free end (Fig. 3). Only once gamonts in biassociative, caudofrontal syzygy were seen (Fig. 4). Primites and satellites resembled each other in shape but not size. Rotational movements, which in gregarines normally mark the onset of encystment (Lange and Wittenstein 2002), were not seen.

Gametocysts (Figs 5-8) were spherical, yellowish or whitish, and variable in size, measuring from 104 to 360 μ m in diameter (mean: 247.7 ± 49.3 ; $n = 50$). A hyaline coat, the ectocyst, of an unusually large thickness of up to 100 μ m was normally evident (Figs 5-

7). The two members of a syzygy were usually still discernable as separate entities in gametocysts found in the midgut or hindgut (Fig. 5). In gametocysts observed in faeces, fusion of members was normally already accomplished (Fig. 6). Mature gametocysts in Petri dishes with moistened filter paper showed the basal discs of the sporoducts, easily seen due to their orange coloration (Fig. 7). The number of basal discs per gametocysts was difficult to determine but appeared to be variable. The maximum number observed was 12. Eversion of sporoducts resulted after further retention of gametocysts under humid conditions (Fig. 8). Sporoducts were basally wide but its length could not be determined.



Figs 9-12. Oocysts and sporozoites of *Leidyana ampulla* sp. n. **9** - disrupted chains of oocysts just dehisced from gametocysts; **10** - isolated oocysts in suspension; **11** - short section of oocyst chain (three oocysts); **12** - a sporozoite emerging from an oocyst (white arrow) and three emerged sporozoites (black arrows). Scale bars 5 μ m (11); 10 μ m (9, 10, 12).

Oocysts (Figs 9-12) were liberated as delicate and easily disrupted chains through the sporoducts of the gametocysts. While oocysts were still in chains they were somehow attached one after the other by their ends (Fig. 11). Oocysts were dolioform and measured 5.7 ± 0.06 by 2.8 ± 0.08 μ m ($n = 50$). Fusiform, mobile sporozoites emerged from oocysts following addition of host digestive extracts (Fig. 12).

Diagnosis

Type host: *Ronderosia bergi* (Stål) 1878 (Orthoptera: Acrididae: Melanoplinae).

Type locality: Surroundings of San Pedro, Misiones province, northeastern Argentina.

Infection site: Epithelium and lumen of gut and gastric caecae.

Trophozoite: Attached to intestinal epithelium, and very rarely free in lumen. Solitary. Slender appearance, with simple, globular epimerite.

Gamont: Bottle-like shape, epimerite retracted into protomerite, considerable variation in size [280-548 (mean: 526.2 ± 13.3) by 104-296 (mean: 223.1 ± 8.5) μ m], solitary, caudofrontal biassociation just prior to syzygy. Primate and satellite of similar shape but not size.

Gametocyst: Spherical, whitish or yellowish, and variable in size (104-360 μ m in diameter, mean: 247 ± 49.3). With up to 12 basal discs of sporoducts.

Oocyst: Uniform in shape (dolioform) and size (5.7 ± 0.06 by 2.8 ± 0.08 μ m).

Deposition of specimens: Type material [gamonts in AFA (alcohol-formalin-acetic acid, Richardson and Janovy 1990) and gametocysts in feces] will be designated and deposited in the collections at the "Center for Parasitological Studies and Vectors (CEPAVE)", La Plata National University, Argentina.

DISCUSSION

By having trophozoites with a simple, globular epimerite, solitary gamonts, gametocyst dehiscence by sporoducts, and dolioform oocysts, the gregarine from *R. bergi* can be clearly assigned to genus *Leidyana*, which was originally established by Watson (1915), and is the single genus in family Leidyaniidae (Clopton 2000). Genus *Leidyana* is very similar in many respects to the much larger genus (in terms of known species) *Gregarina* Dufour (family Gregarinidae) but in the former the association of gamonts is delayed until the onset of syzygy while in the latter gamont association is precocious. Although genus *Leidyana* is acknowledged to be cosmopolitan in distribution (Clopton and Lucarotti 1997), the species found in *R. bergi* is the first one to be recorded in Argentina. Most of the *Leidyana* species known from orthopteroid insects have been recorded as parasites of crickets (Gryllidae) from the old world and North America (Dufour 1837; Watson 1915; Narain 1961; Corbel 1968; Geus 1969; Hoshide 1973, 1978; Haldar and Sarkar 1979; Hooger and Amoji 1986; Sarkar 1988). There is an additional record from a Malagasy cockroach (Blattaria) in North American laboratory colonies (Clopton 1995). Only one other species, *Leidyana subramanii* Pushkala and Muralirangan, has been described from a grasshopper (Acrididae: Eyprepocnemidae), *Eyprepocnemis alacris alacris* (Serville), in Tamil Nadu, India (Pushkala and Muralirangan 1998). Aside from differences based on geographical and host grounds (far apart localities, and different host species belonging to a different subfamily), which would probably suffice as justification for a separate specific status in the present case, the gregarine from *R. bergi* can also be distinguished from *L. subramanii* by its smaller oocysts (5.7 by 2.8 μm vs. 6.6 by 3.5 μm in *L. subramanii*), its epimerite retractile into the protomerite, and the bottle-like appearance of the gamonts. We propose the creation of a new species, *Leidyana ampulla*, for the gregarine in *R. bergi*. The specific name is taken from the Latin *ampulla* ("bottle"),

and refers to the characteristic bottle-like shape of gamonts.

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