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The life cycle of *Gregarina ronderosi* n. sp. (Apicomplexa: Gregarinidae) in the Argentine grasshopper *Dichroplus elongatus* (Orthoptera: Acrididae)

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Abstract

Gregarina ronderosi n. sp. is described based on life cycle observations conducted on nymphs and adults of its natural host, the grasshopper *Dichroplus elongatus*. Following ingestion of oocysts by the host, parasite development occurs between the epithelium and the food mass in the midgut and gastric caeca. Gametocysts are liberated in the faeces. Natural prevalence in the type locality, Girondo, northwestern Buenos Aires Province, was 39.7% ($n = 131$). The earliest trophozoites seen were small ($\leqslant$10 μm), somewhat ovoid, unsegmented bodies. Fully developed trophozoites (the body is divided into epimerite, protomerite, and deutomerite) were slender, with conical or globular epimerites in attached or unattached forms, respectively. Trophozoites varied greatly in size [total length: 10.4–275.1 μm; mean (±S.E.): 126.3 ± 78.9]. Gamonts, which were the most common stages observed and filled the midgut and gastric caeca in grasshoppers kept in rearing rooms, had a stocky appearance and also varied greatly in size (total length: 80–348 μm; 205 ± 13). Association of gamonts was precocious, biassociative, and caudofrontal. Gametocysts were spherical and highly variable in size (96–376 μm in diameter; 202.8 ± 52.5), and normally have 14 sporoduct basal discs. Everted sporoducts were up to 60 μm long. Oocysts were uniformly doliform in shape, measured (5 ± 0.08 by 3.2 ± 0.06 μm) and contained eight sporozoites. Wall reinforcements (carinae) were present. No infection resulted in experimentally inoculated *Locusta migratoria*, which is a host of *Gregarina acridiorum*. *G. ronderosi* is strikingly similar to *G. acridiorum*, but has larger oocysts. © 2002 Elsevier Science (USA). All rights reserved.

Keywords: Eugregarinida; Gregarine; Locust; Protozoa; Septatina

1. Introduction

Dichroplus elongatus Giglio–Tos (Acrididae: Melanoplinae) is the most common and widespread grasshopper in Argentina, and it is also abundant in parts of Chile, Uruguay, and southern Brazil (Cigliano and Lange, 1999). In connection with studies on the biology, natural enemies, and agricultural pest status of *D. elongatus* (Cigliano et al., 1995; De Wysiecki et al., 1997; Lange, 1987, 1997), we often encountered an associated undescribed septate eugregarine (Eugregarinida: Septatina). Previous records of gregarines parasitizing Argentine grasshoppers are mostly anecdotal, with few or no attempts to characterize or identify these parasites (Künckel d'Herculais, 1899; Luna et al., 1981; Marchionatto and Blanchard, 1934; Turk and Barrera, 1979). We describe *Gregarina ronderosi* n. sp. from nymphs and adults of *D. elongatus* to begin to fill in the gap in knowledge about these parasites of Argentine grasshoppers.

2. Materials and methods

During February 1999, nymphs and adults of *D. elongatus* were collected with sweep nets in a grassland site in the vicinity of Girondo, about 20 km southeast of

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Pehuajó in the northwest of Buenos Aires Province. Previous surveys in this area recorded the occurrence of the gregarine. The collected samples were immediately taken to the Center for Parasitological Studies and Vectors (CEPAVE) where the grasshoppers were either frozen at −32 °C ($n = 131$) upon arrival for later examination or were kept in groups in wire-screened cages in a small rearing room under controlled conditions (30 °C, 14L:10D, 40% RH) ($n = 120$) as described by Henry (1985), but with no antibiotics added in the dairy diet. Frozen samples were used for estimating the natural prevalence, the infection intensity and the location of the gregarine within the host. Grasshoppers in cages were maintained for several weeks and individuals were routinely examined in order to conduct the observations.

All grasshoppers, thawed or alive, were examined by ventral, longitudinal dissection under a stereo zoom microscope. Prior to dissection of living insects, a small drop of haemolymph was produced by pulling off a leg. Haemolymph samples were immediately examined as fresh preparations under the compound microscope (400×, 1000×). 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 frass, and transferred to petri dishes containing moistened filter paper (moist or incubation chambers), 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. Emergence of sporozoites was induced by placing oocysts in fresh mounts of host digestive tract extracts as described by Hoshide et al. (1993). A haemocytometer was used for counting oocysts in suspensions (Undeen and Vávra, 1997). The developmental stages, gametocysts, and oocysts from fresh mounts were measured using an ocular micrometer. Measurements of stages are expressed in ranges, means, and standard errors of means. Terminology for the stages of the gregarine follows Levine (1971).

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

Following the protocols of Habtewold et al. (1995) and Lange et al. (2000), third-instar nymphs of healthy *D. elongatus* ($n = 80$) and *Locusta migratoria* (Linn.) ($n = 80$) from CEPAVE's colonies were inoculated *per os* with 10^5 or 10^7 oocysts each, and maintained until death or 30 days, with regular dissections to observe for gregarine development. Third-instar nymphs of healthy *D. elongatus* ($n = 40$) and *L. migratoria* ($n = 40$) were placed in cages for several weeks and held in the same rearing room with cages containing infected *D. elongatus* from Girondo.

3. Results

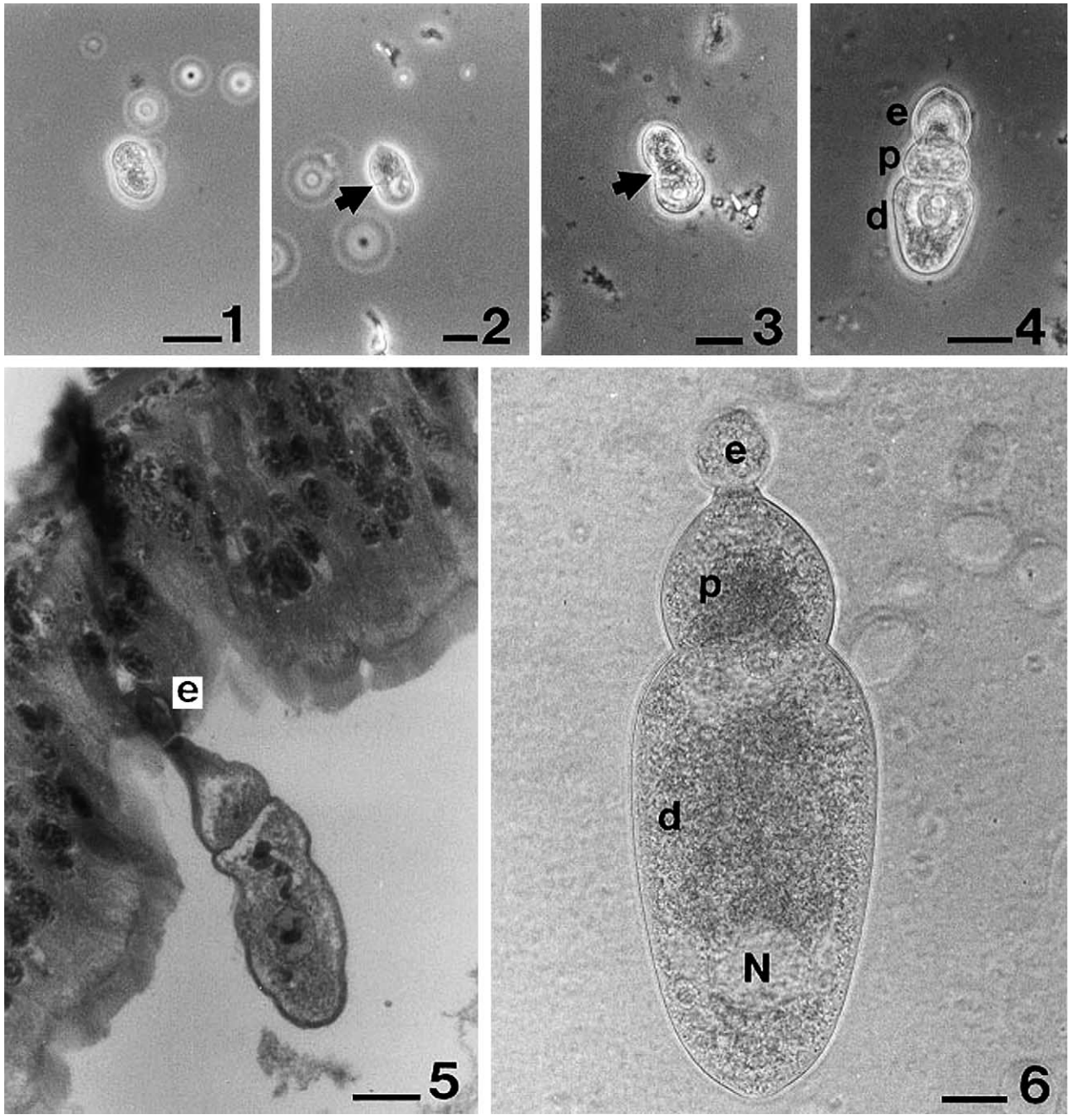
3.1. General observations

The gregarine was detected in 39.7% of the frozen grasshoppers by observation of solitary trophozoites and/or solitary or associated gamonts in the gut and gastric caeca. Gametocysts were observed in the hindgut of two grasshoppers. In contrast, the presence of the gregarine in *D. elongatus* held in cages for prolonged periods of time was heavy, notably as solitary or associated gamonts crowding the midgut (Figs. 7 and 9) and caeca, and gametocysts in hindgut and faeces (Figs. 14 and 19). The gregarine was never detected in the haemolymph samples. The gregarine developed in all inoculated *D. elongatus* but in none of the inoculated *L. migratoria*. All healthy *D. elongatus* placed in cages adjacent to those containing infected insects in the rearing room contracted the parasite while all *L. migratoria* exposed in the same room remained free of the parasite.

3.2. Life cycle

The earliest trophozoites seen were small (⩽10 μm), somewhat ovoidal, unsegmented, uninucleated bodies (Fig. 1) (see cf. Figs. 1–6). Increase in size and gradual development of young trophozoites were accompanied by differentiation of the protomerite–deutomerite septum, with the nucleus remaining in the deutomerite (Figs. 2 and 3). The epimerite of unattached young trophozoites appeared as a disproportionately large structure (Fig. 4) when compared to epimerites of older trophozoites (Figs. 5 and 6). Trophozoites were always solitary (Fig. 6), free in the intestinal or gastric lumen, or attached to the epithelium (Fig. 5). They showed great variation in size, ranging from 10.4 to 275.1 μm in length (Table 1), and were more slender in appearance than gamonts. Epimerites were globular in free trophozoites and mostly conical in attached ones.

Gamonts (Figs. 8 and 10) were the most commonly observed developmental stage. In heavy infections, large, pale-yellow gamonts were easily seen through the



Figs. 1–6. Trophozoites of *Gregarina ronderosi* n. sp. Bars = 10 μm.
Fig. 1. Unsegmented young trophozoite.
Figs. 2 and 3. Young trophozoites showing initial body segmentation (arrows).
Fig. 4. Relatively young trophozoite with body divided into the epimerite (e), protomerite (p), and deutomerite (d).
Fig. 5. Trophozoite attached to the intestinal epithelium of the host showing the conical epimerite (e).
Fig. 6. Free, presumably mature trophozoite showing the globular epimerite (e), protomerite (p), and deutomerite (d) with a large nucleus (N).

midgut wall under the dissecting microscope (Fig. 7) (see cf. Figs. 7–9), and less frequently through the wall of the gastric caeca. They were located between the epithelium and the food contents, apparently following no fixed pattern but most often in a lengthwise position (Fig. 9). Gamonts had a stocky appearance compared to trophozoites and ranged in length from 80 to 348 μm (Table 1). They had a granular cytoplasm, a large nucleus in the deutomerite, and typical epicytic longitudinal folds (Fig. 10) (see cf. Figs. 10–13). The protomerites of gamonts often showed a scar where the epimerite was shed (Figs. 8 and 10). Association of gamonts was early, biassociative, and caudofrontal (Fig. 11). Thc primite and satellite of an association were similar in size and shape,

Table 1
Range and mean (±S.E.) measurements ($n = 30$) in μm of fully differentiated trophozoites (i.e., those showing epimerite, protomerite, and duetomerite), gamonts, primites, and satellites of *Gregarina ronderosi* n. sp.

Character	Range	Mean
Trophozoites		
TL	10.4–2751	126.3 ± 78.9
LE	1–27.5	15.4 ± 0.09
LP	3.1–64.8	31.3 ± 20
LD	6–184.7	78 ± 55
WP	2.4–98.3	38.9 ± 24.2
WD	3.9–114	49.5 ± 27.2
LP:TL	1:3.3–4.2	1:4
WP:WD	1:1.6–1.1	1:1.3
WP:LP	1:1.3–0.65	1:0.8
Gamonts		
TL	80–348	205 ± 13
LP	20–76	49.5 ± 2.5
LD	60–272	155.8 ± 10.8
WP	32–132	76.1 ± 4.7
WD	40–224	115.2 ± 8.8
LP:TL	1:4–4.6	1:4.1
WP:WD	1:0.6–0.6	1:0.6
Primites		
TL	129–360	228.6 ± 12.7
LP	28–88	55.4 ± 2.8
LD	88–272	173.2 ± 10.2
WP	52–120	81.8 ± 3.7
WD	62–232	127.3 ± 8.8
LP:TL	1:4.3–4.1	1:4.1
WP:WD	1:1.2–1.9	1:1.5
WP:LP	1:0.5–0.7	1:0.7
Satellites		
TL	92–356	218.9 ± 12.8
LP	44–72	40.5 ± 1.9
LD	132–310	179.4 ± 11.6
WP	56–120	78.3 ± 4
WD	74–184	113.9 ± 7
LP:TL	1:2.1–4.9	1:5.4
WP:WD	1:1.3–1.5	1:1.4
WP:LP	1:0.8–0.6	1:0.5

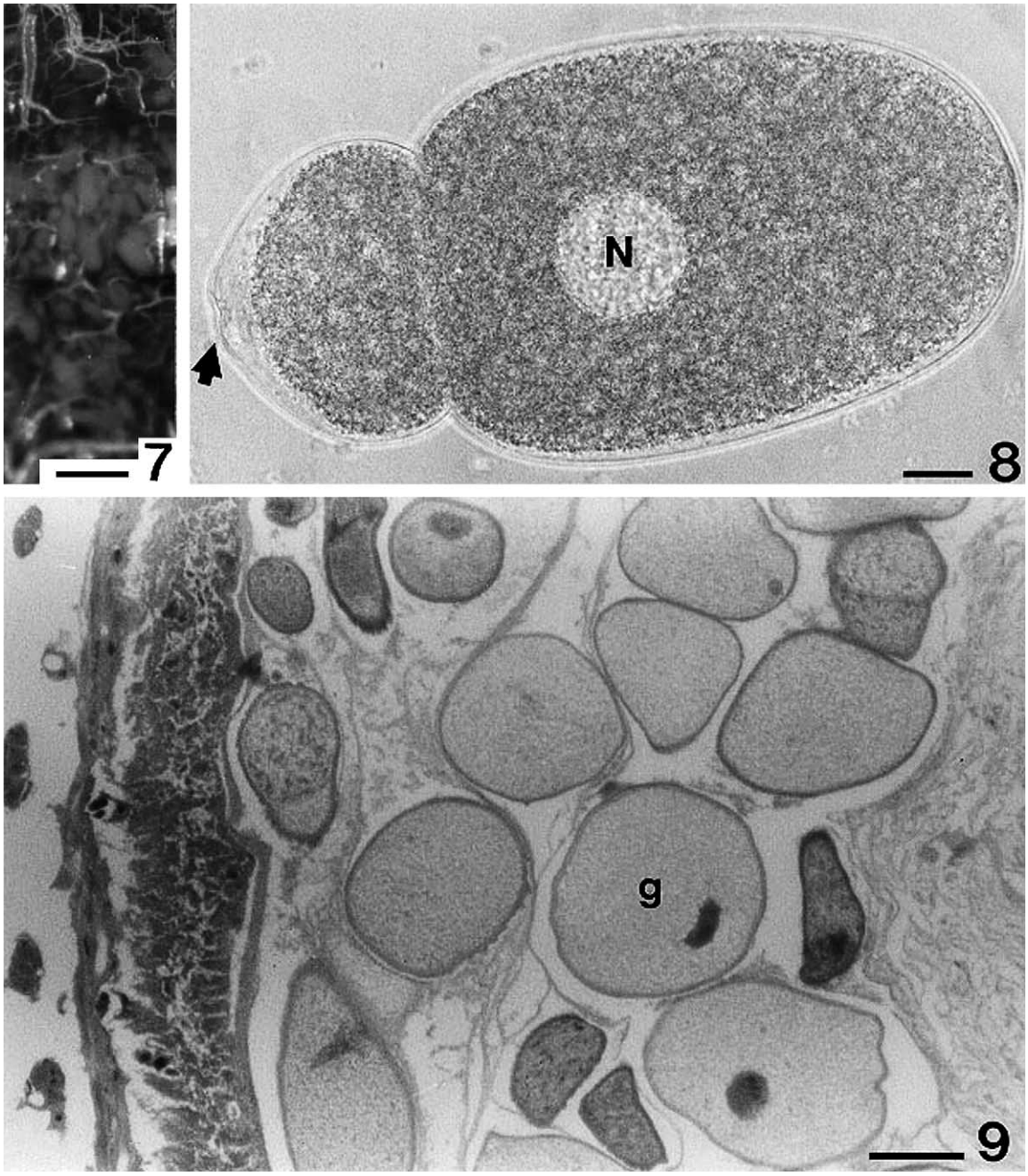
(TL) Total length; (LE) length of epimerite; (LP) length of protomerite; (LD) length of deutomerite; (WD) width of protomerite; (WD) width of deutomerite.

but there was considerable variation in size between associations. Although gamonts in association were very common, rotational movements, marking the onset of encystment, were rarely seen (Figs. 12 and 13). The few rotating pairs of gamonts that were observed did not complete encystment. Sometime early in the rotational event the protomerite–deutomerite septa of primite and satellite "disappeared" and there were conspicuous changes in the relative positioning of the nuclei originally located in the deutomerites.

Gametocysts were spherical, whitish, and highly variable in size. They measured from 96 to 376 μm in diameter (mean = 202.8 ± 52.5; $n = 50$). A thick, translucent hyaline coat, the ectocyst, was present (Figs. 14 and 15) (see cf. Figs. 14–19). Associated gamonts in gametocysts found in the midgut or hindgut were usually in a separate condition (Figs. 14 and 16). In gametocysts observed in faeces, fusion of associates was normally complete. After being held for as little as 24 h in a humid chamber, the orange basal discs of the sporoducts began to appear on the surfaces of gametocysts (Fig. 17). Basal discs appeared to be distributed evenly on the surface of gametocysts. The number of basal discs counted per gametocyst was variable, ranging from 12 to 15 but with most gametocysts (60%; $n = 50$) having 14. Further retention of gametocysts under humid conditions resulted in the eversion of sporoducts through the layers of the ectocyst (Fig. 18). Everted sporoducts were wider basally and measured as much as 60 μm long. The chains of oocysts in an end to end arrangement dehisced through sporoducts. The chains were extremely long and became entangled when several gametocysts underwent dehiscence simultaneously (Fig. 19). A delicate membrane or sheath appeared to contribute to the maintenance of oocysts in the chain (Fig. 21). Oocysts were remarkably uniform in shape and size (Figs. 20–25). They were ovocylindrical with somewhat blunted ends (doliform- or barrel-shaped) and measured 5 ± 0.08 μm by 3.2 ± 0.06 μm ($n = 50$). Inducing the emergence of the mobile, vermiform sporozoites proved to be relatively easy when host digestive tract extracts were used as an induction solution (Figs. 22 and 23). The maximum number of sporozoites counted per oocyst was eight. Scanning electron photomicrographs revealed that oocysts appeared to have some structural reinforcements (carinae) in the cyst wall with the shape somewhat reminiscent of a wishbone (Figs. 24 and 25).

4. Discussion

Early biassociative gamonts, simple globular or conical epimerite, dehiscence of oocysts by sporoducts, and doliform oocysts place the gregarine isolated from *D. elongatus* in the genus *Gregarina* as defined by Dufour (1828) and revised by Watson (1916), Kamm (1922), and Levine (1988). This is the first *Gregarina* sp. associated with Argentine Acrididae to be described based on complete life cycle data. Previous records were circumstantial or fragmentary observations that did not include characterizations or identifications, although there is one described species, *Gregarina paranensis* (Künckel d'Herculais, 1899). This species was reported occurring in a different host in the subfamily Cyrtacanthacridinae, the locust *Schistocerca cancellata* (Serville) from northwestern Argentina. Information that would have allowed comparison with the species form *D. elongatus* was not provided. We were not able to find *G. paranensis* for a comparative study. Until this



Figs. 7–9. Gamonts of *Gregarina ronderosi* n. sp.

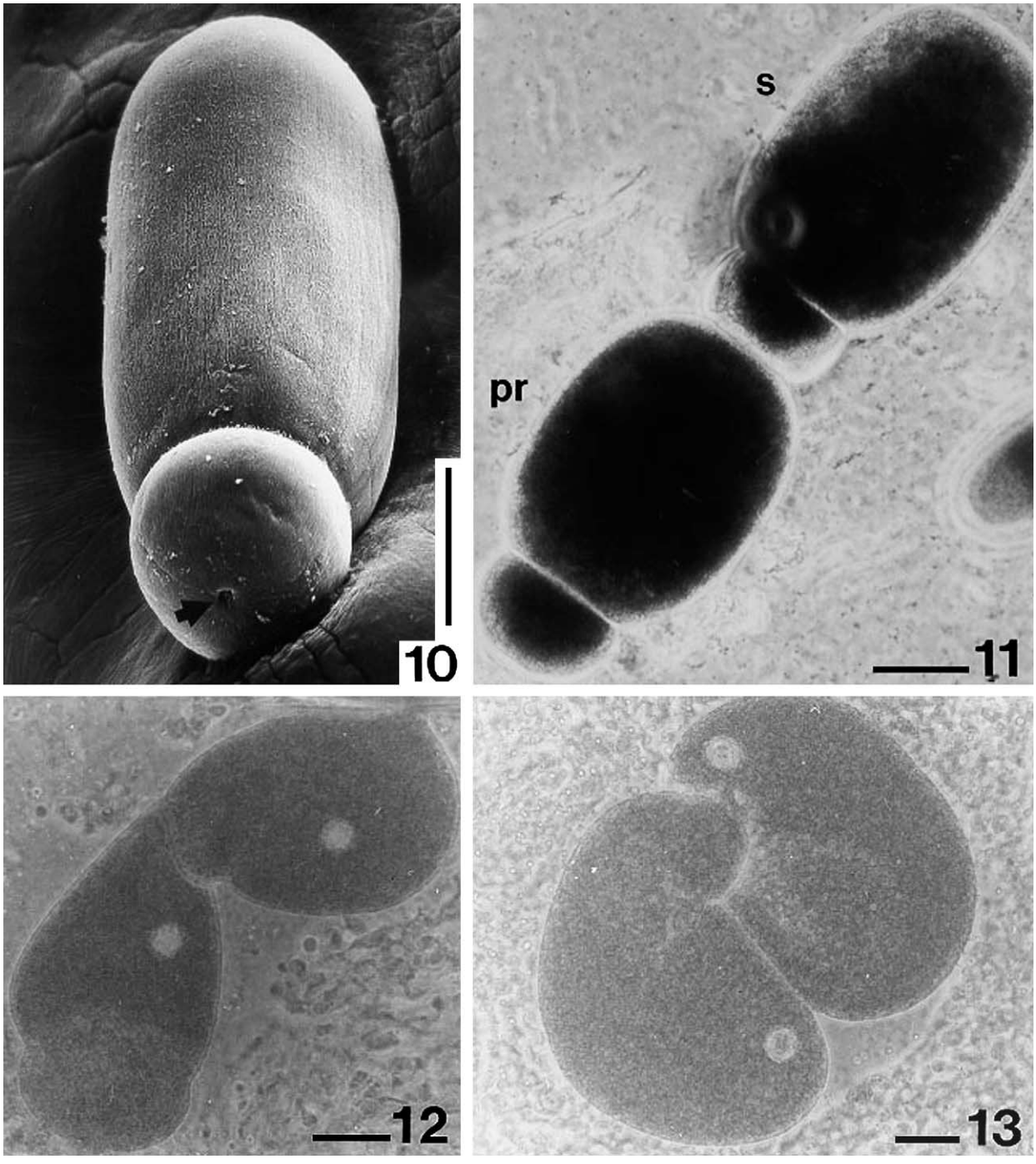
Fig. 7. Gamonts observed through intestinal wall of host. Bar = 300 μm.

Fig. 8. Gamont showing granular cytoplasm, large nucleus (N) in deutomerite, and a scar (arrow) in the protomerite where the epimerite was shed. Bar = 17 μm.

Fig. 9. Cross-sections of gamonts (g) in the intestinal lumen. Bar = 50 μm.

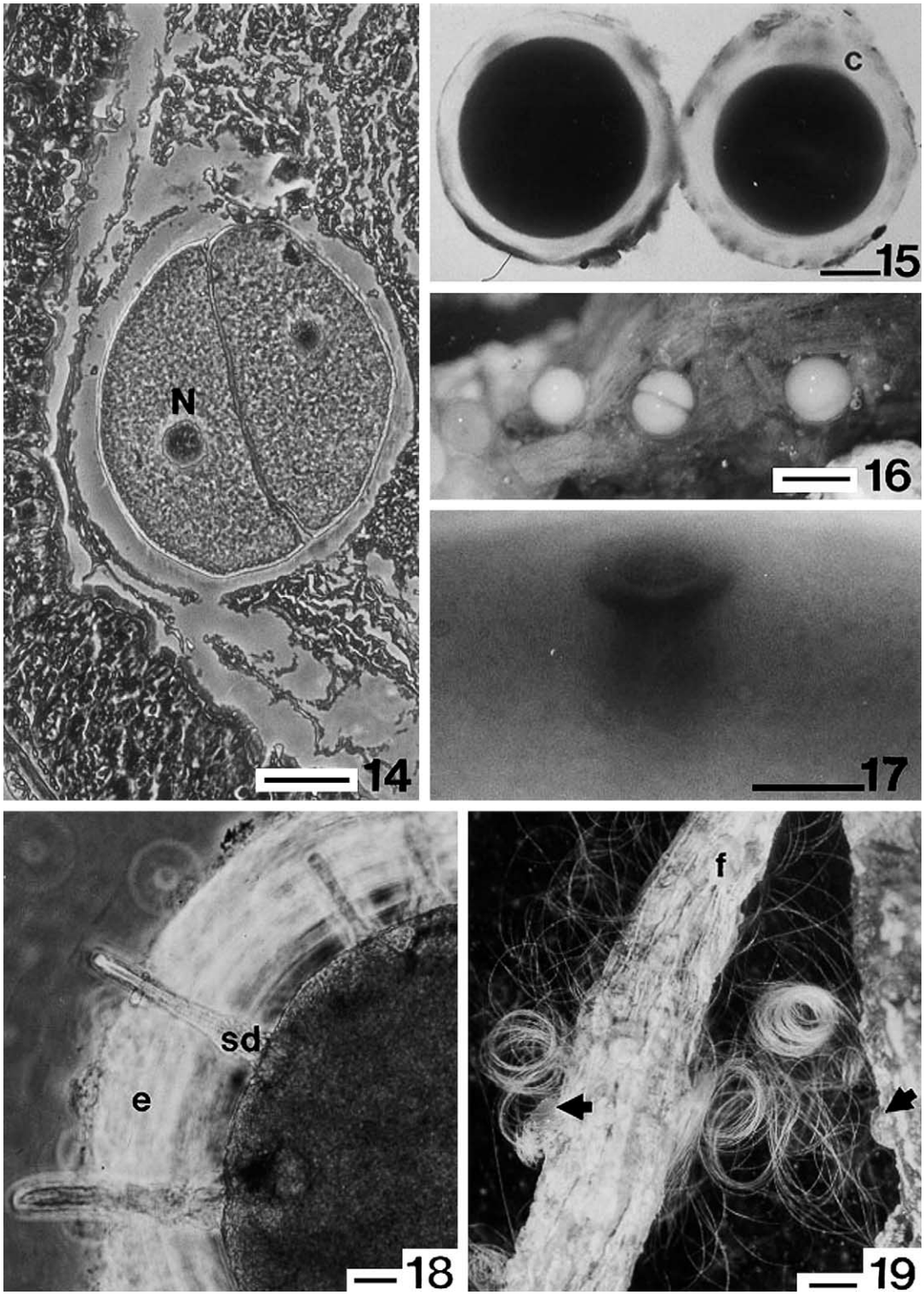
species is found we presume on the basis of host identity and geography that the gregarine in our study is a different species.

The last review on the genus *Gregarina* (Levine, 1988) listed 298 species, 13 of which were isolated from grasshoppers or locusts (Acrididae): *Gregarina acantholobi* Hoshide, *Gregarina acridiorum* Léger, *Gregarina acrydiinarum* Semans, *Gregarina boevi* Corbel, *Gregarina desaigeri* Théodorides, Ormieres, and Jolivet *Gregarina garnhami* Canning, *Gregarina inago* Hoshide, *Gregarina indianensis* Semans, *Gregarina locustae* Lankester, *Gregarina nigra* Watson, *G. paranensis* Künckel d'Herculais, *Gregarina poeciloceri* Ganapati and Mrutyunjayedevi, and *Gregarina rigida* (Hall) Ellis. Recent work reduced the number of species to 10. Lipa et al. (1996) reported that *G. garnhami*, the most studied



Figs. 10–13. Gamonts of *Gregarina ronderosi* n. sp. Bars = 50 μm.
Fig. 10. Gamont showing longitudinal folds of epicyte scar (arrow) in protomerite.
Fig. 11. Gamonts in caoudofrontal syzygy: (pr) primite, (s) satellite.
Figs. 12 and 13. Associated gamonts in rotation.

species, is actually a junior synonym of *G. acridiorum*. Kula and Clopton (1999) demonstrated that *G. indianensis* and *G. nigra* are conspecific and do not qualify for inclusion in genus *Gregarina*. A new genus, *Amoebogregarina*, was erected with *A. nigra* as the type species. In originally describing the gregarine species associated with Acrididae, researchers relied heavily on the ''different host, different parasite'' concept because distinguishing species of *Gregarina* solely on morphological grounds is problematic due to an apparent lack of reliable diagnostic characters. The use of morphometrics was also of little help because very similar values in ranges and ratios are found between species. Species descriptions were not based on characterizations of en-



Figs. 14–19. Gametocysts of *Gregarina ronderosi* n. sp.

Fig. 14. Section of a gametocyst in intestinal lumen in which associates are still separated and showing one nucleus (N) each. Bars = 50 μm.

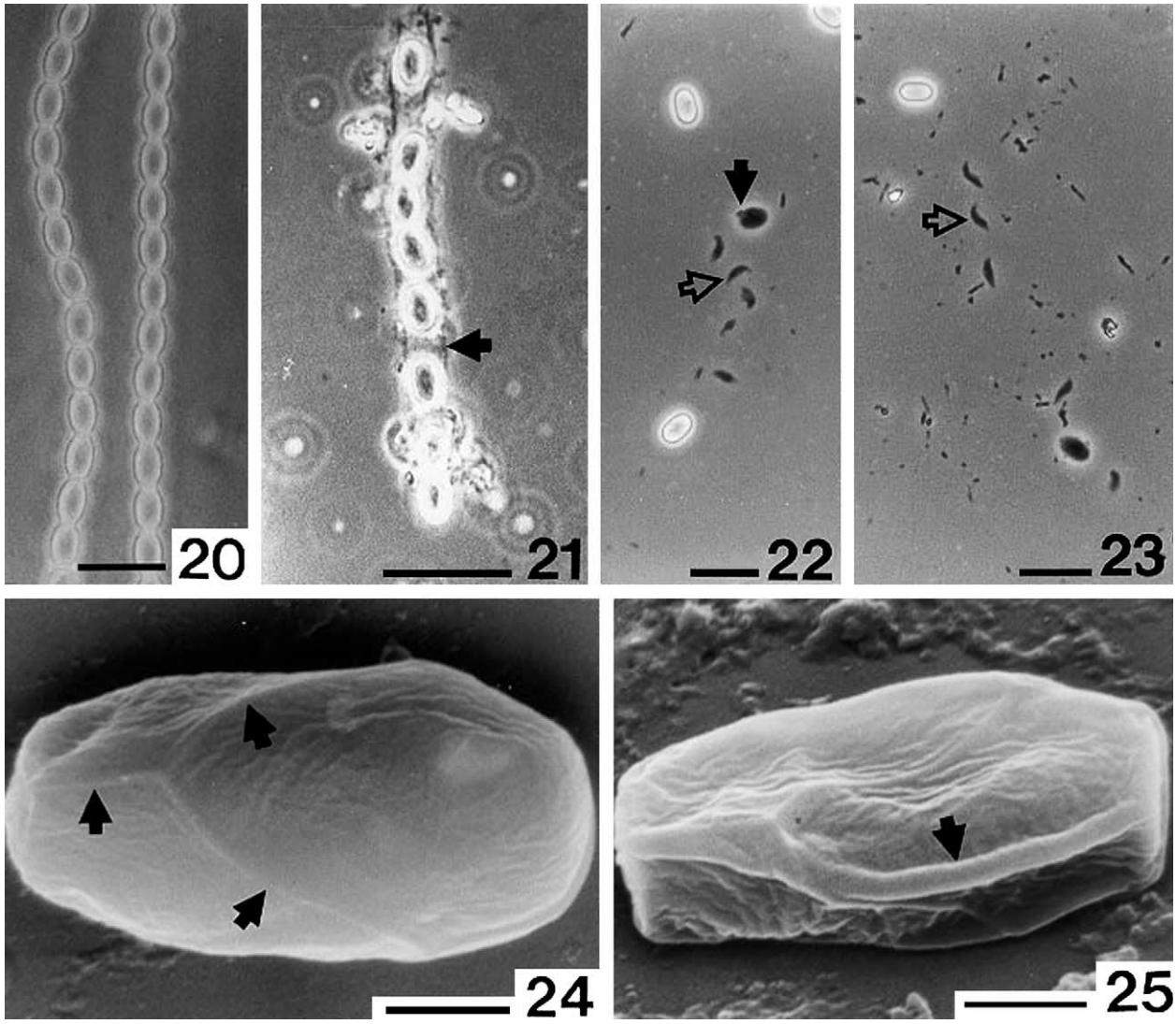
Fig. 15. Two gametocysts showing relative thickness of ecotocytes (c). Bars = 50 μm.

Fig. 16. Gametocysts encased in host's intestinal food mass, the one in the centre having the associates still in a separate condition. Bars = 200 μm.

Fig. 17. Basal disc of sporoduct. Bars = 10 μm.

Fig. 18. Everted sporoducts (sd) through the ectocyte (e) of a gametocyst. Bars = 10 μm.

Fig. 19. Entangled chains of oocysts dehisced from gametocysts (arrows) in faeces (f). Bars = 200 μm.



Figs. 20–25. Oocysts and sporozoites of *Gregarina ronderosi* n. sp.
Fig. 20. Chain of oocysts in end to end arrangement. Bar = 10 μm.
Fig. 21. Part of a chain of oocysts. A delicate sheath enclosed the oocysts (arrow). Bar = 10 μm.
Figs. 22 and 23. Oocysts, emerged (empty arrows), and emerging (solid arrow) sporozoites. Bars = 10 μm.
Figs. 24 and 25. Oocysts and collapsed oocyst showing wishbone-shaped wall reinforcements (arrow). Bars = 1 μm.

tire life cycles but using few or just a single individual host. Often a gamont, usually the most commonly found stage in naturally infected grasshoppers, was the only life stage described. Using the available information, the *Gregarina* species associated with Acrididae are so similar morphologically that, if host and geographical consideration were left aside, it would probably be possible to include the new gregarine of *D. elongatus* in almost any of the described species. Nevertheless, host and geography considerations carry an increased relevance for the newly discovered *Gregarina* because, with the exception of *G. paranensis*, all known species of *Gregarina* in Acrididae have been described from grasshopper species of the old world and North America. The only *Gregarina* species of Acrididae that has been studied in some detail was *G. acridiorum* from laboratory-reared locusts, (*Schistocerca gregaria* (Forskål), *Locusta migratoria*, and *Anacridium aegyptium* (Linn.), in England (Canning, 1956; Harry, 1965, 1970; Walker et al., 1979). Aside from some morphometric differences (size of trophozoites, gamonts, and gametocysts) that we believe are inconclusive for delimiting species, there is a striking similarity of general developmental and morphological characteristics between *G. acridiorum* and the *Gregarina* in *D. elongatus*. However, both species appear to be distinguishable by the number of basal discs of sporoducts per gametocyst (eight in *G. acridiorum*, normally 14 in *Gregarina* from *D. elongatus*) and the larger size of oocysts of *G. acridiorum* (6.5–7 × 4 μm) relative to those of *Gregarina* from *D.*

elongatus (5×3.2 μm). Since we were not completely satisfied by these differences for taxonomic purposes we looked for additional evidence by trying to induce infections either by standard oral administration of oocysts or by placing infected host insects in prolonged and close contact with *L. migratoria*, a potential and known host of *G. acridiorum*. The negative results obtained (i.e., lack of infection development) support the separate specific status of *G. acridiorum* and the gregarine in *D. elongatus*. As a group, species of *Gregarina* are well known for their easiness of horizontal transmission to susceptible insects in experimental work or when they are held as colonies in captivity (Brooks and Jackson, 1990; Henry, 1985). Given the great similarity of the *Gregarina* spp. associated to Acrididae, it would probably be very informative to study at the molecular level as many species as possible. Until such a possibility becomes feasible, and based on the characteristics of the eugregarine commonly occurring in the digestive tract of *D. elongatus*, the erection of a new species, *G. ronderosi*, is proposed. The specific name is in honour of Dr. Ricardo A. Ronderos, who was a prominent Argentine acridologist. Diagnosis is as follows.

4.1. Gregarina ronderosi n. sp.

Type host. Dichroplus elongatus Giglio–Tos, 1894 (Orthoptera: Acrididae: Melanoplinae).

Type locality. Girondo, 20 km southeast of Pehuajó, Buenos Aires Province, Argentina.

Infection site. Midgut and gastric caeca, between epithelium and food mass.

Trophozoite. Attached or unattached to gut epithelium, solitary, great variation in size [10.4–275.1 (mean: 126.3 ± 78.9) by 3.9–114 (49.5 ± 27.2) μm], slender appearance. Epimerite globular in unattached trophozoites and conical in attached trophozoites.

Gamont. Stocky appearance compared to trophozoites, great variation in size [80–348 (mean: 205 ± 13) by 40–224 (115.2 ± 8.8) μm]. Protomerites often showing a scar where epimerite was shed. Early association, biassociative, and caudofrontal. Primite and satellite of similar size and shape.

Gametocyst. Spherical and highly variable in size (96–376 μm in diameter, mean: 202.8 ± 52.2). Normally with 14 basal discs of sporoducts.

Oocyst. Highly uniform in shape (doliform) and size (5 ± 0.08 by 3.2 ± 0.06 μm. With eight vermiform, uninucleate sporozoites, and structural reinforcements (carinae) in the wall.

Deposition of specimens. The holotype slide (1414L) has been deposited in the author's collection at the "Centro de Estudios Parasitológicos y de Vectores (CEPAVE)," La Plata National University, Argentina. A paratype series has been deposited in the Harold W. Manter Laboratory for Parasitology (HWML), Division of Parasitology, University Nebraska State Museum, Lincoln, Nebraska.

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