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## *Mattesia geminata* sp. n. (Neogregarinida: Ophrocystidae) a Parasite of the Tropical Fire Ant, *Solenopsis geminata* (Fabricius)\*

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**SYNOPSIS.** A new species of neogregarine, *Mattesia geminata* sp. n., that infects immature stages of the tropical fire ant, *Solenopsis geminata* (Fabricius), is described. The parasite, which develops in the hypodermis, causes disruption of the developing eyes, melanization of the cuticle, and death of pupae. Intracolony infection rates are usually less than 2%, but may exceed 90%. Attempts to transmit the infection were unsuccessful.

**Index Key Words:** *Mattesia geminata* sp. n.; Neogregarinida; fire ant, *Solenopsis geminata*.

**T**HE red and black imported fire ants, *Solenopsis invicta* Buren and *Solenopsis richteri* Forel, are medical and agricultural pests in the Southeastern United States. Consequently, reports of microsporidan infections in these ants in their homelands in South America (1, 2) stimulated interest in research on their biologic control. Surveys for pathogens subsequently revealed virus-like particles (3) and several additional

microsporida (8) infecting various *Solenopsis* species in South America and the United States. We describe here a neogregarine that parasitizes the tropical fire ant, *Solenopsis geminata* (Fabricius), a species native to the Southern United States.

### MATERIALS AND METHODS

**Light Microscopy.**—Stained smears of larvae and pupae were prepared by air drying the slide, fixing in methanol for 3-5 min, staining with 10% Giemsa in phosphate-buffered distilled water (pH 7.41) for 10 min, and rinsing with tap water. To determine the number of nuclei in each spore, methanol-fixed smears were

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hydrolyzed in warm (70 C) 1 N HCl for 15-30 sec before staining (mature spores do not readily absorb Giemsa or iron hematoxylin stains). Paraffin sections were prepared by fixing specimens in Carnoy's fixative overnight, embedding in paraffin, and staining with iron hematoxylin and eosin.

Living spores were measured with an A. E. I. Cook image-splitting micrometer at a magnification of 1,000 $\times$ .

**Scanning Electron Microscopy.**—Spores were fixed in 1% (w/v) OsO<sub>4</sub>, dehydrated in ethanol series, dried in a critical point drier, and coated with gold. Electronmicrographs were taken in a scanning electron microscope at an accelerating voltage of 20 kV.

**Transmission Tests.**—*Per os* transmission tests were conducted by feeding small healthy colonies of *S. geminata* boiled egg yolks wetted with aqueous suspensions of spores prepared from either fresh or dried (up to 3 months) dead pupae, or from living pupae. This technic has been successful for *per os* transmission of microsporidia in fire ants (7). In addition, transmission was attempted by placing diseased pupae in healthy colonies (conspecific pupae are adopted). Culture of the parasite was also attempted in a factitious host, the corn earworm, *Heliothis zea* (Boddie).

Collection, maintenance, and screening of ant colonies was as described previously (8).

#### DESCRIPTIVE ACCOUNT AND DISCUSSION

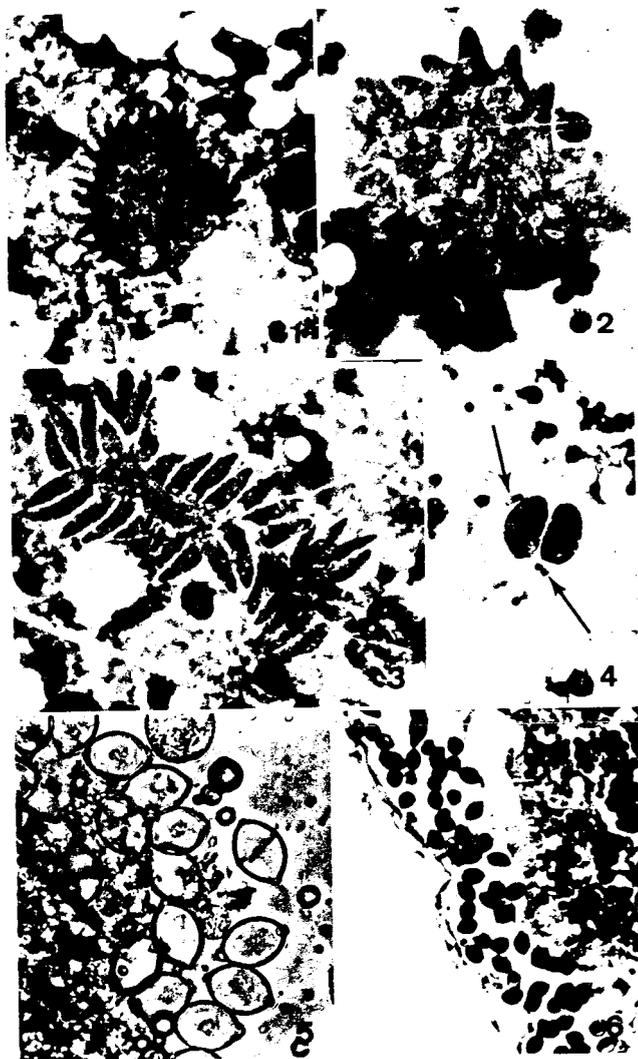
The genus *Mattesia* Naville is characterized by cycles of micronuclear and macronuclear merogony, and by gametogony resulting in the formation of 2 spores within a gametocyst. These definitive stages are shown for *Mattesia geminata* sp. n. in Figs. 1-4. All stages of the life cycle depicted by Canning (4) in her study of *Mattesia trogodermae* Canning and by McLaughlin (9) in his study of *Mattesia grandis* McLaughlin have been seen in *M. geminata*.

Living spores of *M. geminata* measure 7.9 (7-8.5)  $\times$  11.3 (10.5-12.5)  $\mu$ m and are lemon-shaped, obese ovals with a protuberance at each pole (Fig. 5). By SEM each polar protuberance is seen to consist of 2 annular ridges and a plug. The remainder of the spore is without surface sculpture. When examined with the aid of phase-contrast optics, the polar plugs are seen to protrude into the interior of the spores (Fig. 5). Mature spores contain 8 randomly distributed nuclei.

The gametocyst membrane of *M. geminata* appears to be subsupersistent or fragile; consequently, even immature spores are rarely observed in pairs.

The earliest pathologic manifestation of *M. geminata* infection is irregularity of the developing eyes of the pupa. This is followed by melanization of the cuticle, which begins in the legs and posterior margins of the sclerites of the gaster. Ultimately, the entire pupa becomes almost solid black (Fig. 7). Paraffin sections of such pupae contain spores of the pathogen concentrated in the area of the hypodermis; most of the hypodermal tissue has been destroyed (Fig. 6). Pupae with these manifestations do not mature, and we have not detected the parasite in adults from infected colonies. Thus, the disease appears to be highly fatal.

The intracolony infection rates are usually less than 2%; however, in 3 colonies the infection rates exceeded 90%. All but one of the infected colonies were found at the type locality, a roadside  $\sim$ 2 km in length. Over a period of 2 years, more than 25 colonies ( $\sim$ 20% of the total colonies) collected at this site were infected. Only one in a sample of 307 colonies from 74 other sites in Florida and Georgia was infected (8). We have seen similar spores from an unidentified *Solenopsis* sp. (not *S. geminata*) from Brazil. On several occasions, individual pupae having dual infections with *M. geminata* and the microspo-



Figs. 1-6. [Photomicrographs of developmental stages of *Mattesia geminata* sp. n. from *Solenopsis geminata* (Fabricius). Figs. 1-5, smears. Figs. 1-4, Giemsa stain.  $\times$ 750.] 1. Micronuclear plasmodium. 2, 3. Early (Fig. 2) and late (Fig. 3) macronuclear plasmodia. 4. Gametocyst with binucleate sporoblast and residual bodies (arrows). 5. Living mature spores. Phase contrast.  $\times$ 900. 6. Spores in the hypodermis seen in a paraffin section. Carnoy's fluid, iron hematoxylin-eosin.  $\times$ 300.

ridan, *Burenella dimorpha* Jouvenaz & Hazard, have been found.

All attempts to transmit the infection to healthy colonies of *S. geminata* failed. We do not know whether particular conditions are necessary for germination of *M. geminata* spores or whether *S. geminata* is a factitious host in which only non-infectious spores are produced. Spores dried for up to 3 months were noninfectious *per os*. It has not yet been possible to examine adequately the large and varied arthropod fauna associated with fire ants for infection. Collins & Markin (5) listed 52 species of insects which have been collected from fire ant nests. The immature forms of these inquilines are particularly hard to collect, and many are undescribed. Corn earworm lar-



Fig. 7. Living pupae of *S. geminata*. Pathologic manifestations are evident in pupae in the left and center. The pupa on the left is normal.  $\times 20$ .

vae were also refractory to infection with *M. geminata*. An attempt to infect *S. geminata* with *M. trogodermae* by feeding the ants infected *Trogoderma glabrum* (Herbst) (Coleoptera) larvae and spores in boiled egg yolk also failed.

Six species of *Mattesia* have been previously described. Three of these, *M. trogodermae*, *M. grandis* (10), and *Mattesia oryzaephili* Ormieres, Louis & Kuhl (12), parasitize Coleoptera; 2, *Mattesia dispora* Naville (11) and *Mattesia povolnyi* Weiser (13), parasitize Lepidoptera; and one, *Mattesia bombi* Liu, Macfarlane & Pengelly (9), parasitizes Hymenoptera (bumblebees). A neogregarine parasite of fleas was named *Mattesia orchopiae* by Dasgupta (6); however, it is monosporous, and therefore Canning (4) reassigned it to the genus *Coelogregarina*. *Mattesia geminata* can be differentiated from other *Mattesia* spp. by spore size and shape, prominence of polar protuberances, host, and tissue specificity. Also, the gametocyst membrane of *M. geminata* appears to be relatively fragile.

#### TAXONOMIC SUMMARY

##### *Mattesia geminata* sp. n.

**Diagnosis.**—Living Spores:  $7.9 (7-8.5) \times 11.3 (10.5-12.5) \mu\text{m}$ , obese oval with protuberance at each pole; surface sculpture lacking; octonucleate; stain poorly with Giemsa and iron hematoxylin; disporous, but gametocyst membrane subsistent or fragile; 2 residual bodies associated with paired sporoblasts in gametocysts. **Vegetative Stages:** micronuclear and macronuclear plasmodia with deeply staining cytoplasm (Giemsa), micronuclei  $2 \mu\text{m}$ , macronuclei  $4-5 \mu\text{m}$  (Giemsa); nuclei of plasmodia and merozoites with eccentric endosome.

**Host.**—Immature stages of the tropical fire ant, *Solenopsis geminata* (Fabricius).

**Infection Site.**—Hypodermis, apparently in oenocytes.

**Holotype.**—Gainesville, Alachua Co., Florida, U.S.A. USNM No. 24546.

**Paratype.**—As for holotype. USNM No. 24547.

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