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New Family, Genus, and Species of Microsporida (Protozoa: Microsporida) from the Tropical Fire Ant, *Solenopsis geminata* (Fabricius) (Insecta: Formicidae)*

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SYNOPSIS. A new species of Microsporida, *Burenella dimorpha* sp. n., representing a new family, Burenellidae fam. n. and genus, is described on the basis of light- and electron-microscope observations. The family is characterized by 2 sequences of sporogony, each sequence having morphologically different sporonts and spores. The parasite infects the tropical fire ant, *Solenopsis geminata* (Fabricius), producing distinct pathologic manifestations (clearing of the cuticle and eye malformation) and death in the pupal stage of development. Transmission of the infection *per os* to healthy *S. geminata*, to the Southern fire ant, *Solenopsis xyloni* McCook, and to the red and black imported fire ants, *Solenopsis invicta* Buren and *Solenopsis richteri* Forel, is reported.

Index Key Words: *Burenella* gen. n.; *Burenella dimorpha* sp. n.; Burenellidae fam. n.; Microsporida; light microscopy; electron microscopy; taxonomy.

THE first microsporidan infection in ants was discovered during a taxonomic study of the red imported fire ant, *Solenopsis invicta* Buren (1). While examining alcohol-preserved specimens from Mato Grosso, Brazil, Buren observed subspherical cyst-like bodies which contained microsporidan spores in the gasters of worker ants. This parasite has been described from fresh material by Knell et al. (5) as a new species of *Telohania*.

Subsequently, Allen & Silveira-Guido (2) reported similar microsporida from the black imported fire ant, *Solenopsis richteri* Forel, in Uruguay and Argentina, and from an unidentified *Solenopsis* sp. in Uruguay. These findings stimulated interest in biologic control of imported fire ants, which are medical and agricultural pests in the Southeastern United States. Efforts to control these ants through chemical means have stirred a controversy which has raged for more than a decade (7). Therefore, surveys for pathogens of fire ants are being conducted in South America and in the United States. We describe here a

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microsporidium that infects the tropical fire ant, *Solenopsis geminata* (Fabricius), and which represents a new genus. It is similar in its development to *Vairimorpha necatrix* (Kramer), and we believe these microsporida constitute a new family. We report *per os* transmission of the new microsporidan in *S. geminata*, *S. invicta*, *S. richteri*, and the Southern fire ant, *Solenopsis xyloni* McCook.

MATERIALS AND METHODS

Light Microscopy.—Intact immature and adult ants were examined with a dissecting microscope for symptoms of disease. Fresh smears of symptomatic and mass extracts of asymptomatic specimens were examined by phase microscopy at a magnification of 600 \times . Smears of specimens suspected of being infected were prepared by air drying the slide, fixing in methanol for 3-5 min, staining with 10% Giemsa in phosphate-buffered (pH 7.41) distilled water for 10 min, and rinsing with tap water. Stained smears were prepared also by smearing infected specimens on a glass coverslip, immersing immediately in Bouin's fixative for ~ 6 h, washing with 70% ethanol, staining overnight in 6.5% (w/v) aqueous solution of hematoxylin, destaining with iron alum to desired intensity (Heidenhein's-type method), dehydrating, and mounting on a microscope slide. Paraffin sections were prepared by fixing specimens in Carnoy's fixative overnight, imbedding in paraffin, and staining with Heidenhein's hematoxylin and eosin.

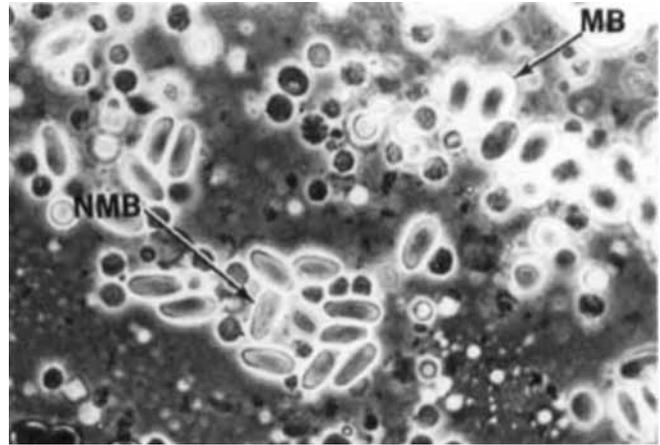
Living spores were measured with an A. E. I. Cook image-splitting micrometer at a magnification of 1,000 \times .

Electron Microscopy.—Tissues were dissected and fixed with 4% (v/v) glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 24 h at 8 C and post-fixed in 1% (w/v) OsO₄ in 0.1% (w/v) cacodylate buffer for 23 h at room temperature. Tissues were then block-stained in 2% (w/v) aqueous uranyl acetate, dehydrated in ethanol, and embedded in Spurr's (11) low viscosity medium. Thin sections were stained in aqueous uranyl acetate and Reynold's (10) lead citrate. Electronmicrographs were taken in a Hitachi 125-E electron microscope at an accelerating voltage of 50 kV.

Transmission Tests.—*Per os* transmission tests were conducted by feeding 3 small healthy colonies of *S. geminata* and *S. invicta*, 2 similar colonies of *S. richteri*, and one similar colony of *S. xyloni* with boiled egg yolk wetted with an aqueous suspension of spores daily for 3 days. That these colonies were originally healthy was determined by triturating 80-90% of the adults and immatures of each laboratory colony in a glass tissue homogenizer with water sufficient to just cover the mass of ants, and then examining the aqueous extract for spores. Tests of mixtures of diseased specimens of *S. geminata* and healthy specimens of *S. invicta* had indicated that an infection rate of less than 0.1% can be detected by this method. The remaining 10-20% of the workers and brood, together with the queen, were maintained in small plastic nests similar to those described by Wilson (12). They were fed on a diet consisting of macerated laboratory-reared insects, pureed beef, and raw eggs in agar supplemented with once-refined soybean oil. Pupae were examined for pathologic manifestations, vegetative stages, and spores ~ 3 weeks after exposure to spores.

DESCRIPTIVE ACCOUNTS AND DISCUSSION

This new genus name, *Burenella* gen. n., is proposed for a microsporidan parasite *S. geminata* having 2 sequences of sporogony that produce morphologically different sporonts and spores (Figs. 1-5). One sequence has disporous sporonts that sporulate



All figures are of *Burenella dimorpha* gen. n., sp. n.

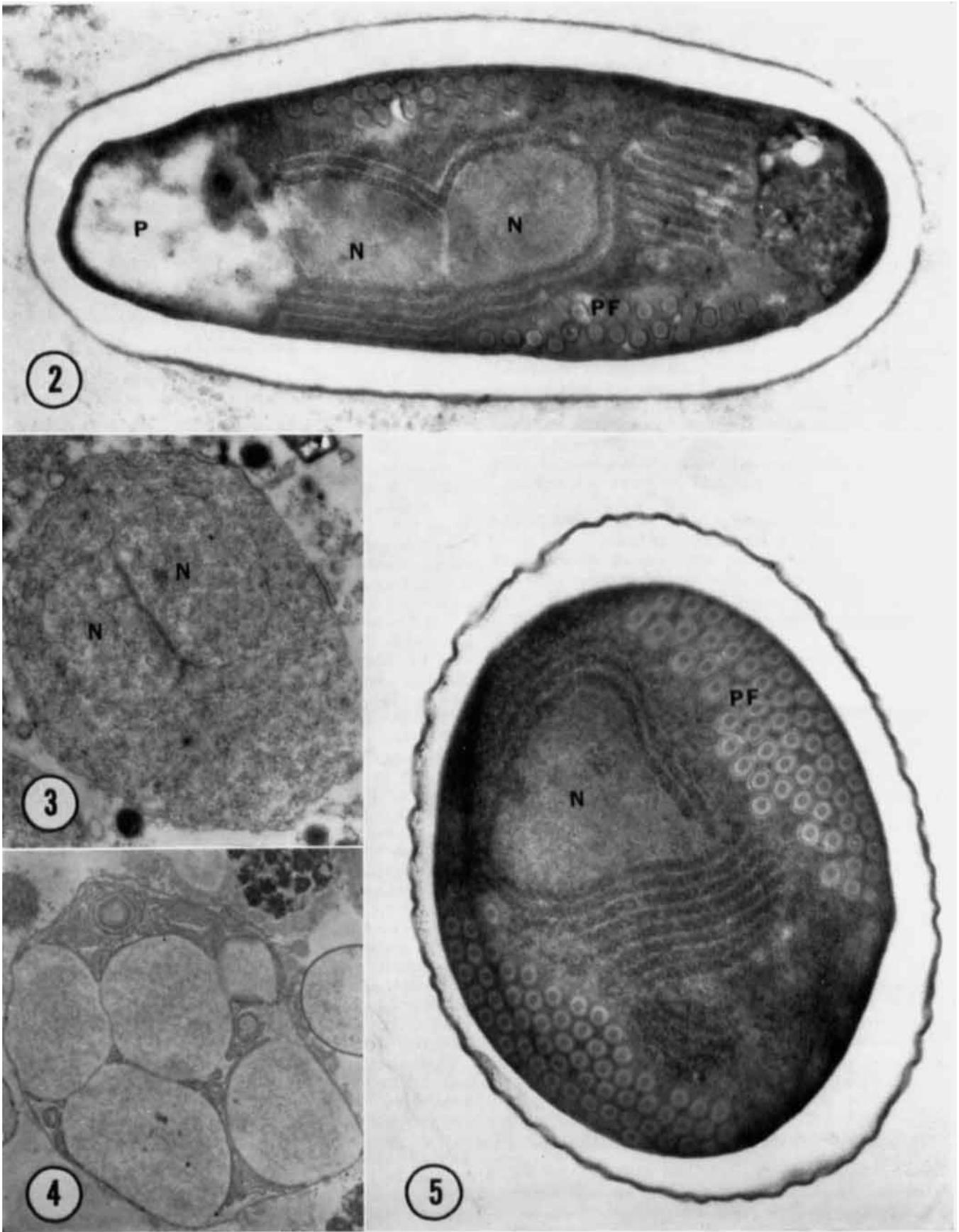
Fig. 1. Living nonmembrane-bounded (NMB) spores and membrane-bounded (MB) spores. $\times 1,000$.

in the hypodermis, producing binucleate, non-membrane bounded (NMB) spores. The 2nd sequence has multinucleate sporonts that produce 8 uninucleate, membrane-bounded (MB) spores in fat cells (Fig. 6). The membrane (pansporoblastic membrane) enclosing the spores in the latter sequence is not persistent. Consequently, mature MB spores are not seen in octets by light microscopy since the membrane ruptures when the host is dissected.

NMB spores are coniform, being broadly rounded posteriorly and slightly constricted anteriorly; MB spores are broadly oval (Fig. 1). The polar filament of both spores is nearly uniform in diameter. In the MB spore of the single known species it is extremely long, having ~ 57 coils; in the NMB spore it has only ~ 26 coils. The structures of the polaroplasts are indistinct in both spores. The exospores are thin, the endospores thick, and the surfaces of both spores are smooth.

Certain genera of the family Thelohaniidae Hazard and Oldacre 1975 exhibit spore dimorphism. *Burenella* is not related to these but does appear to be related to another dimorphic microsporidan, formerly placed in the genus *Nosema* (*N. necatrix* Kramer). Kramer (6) observed both MB and NMB spores in the armyworm, *Pseudoletia unipuncta* (Haworth), and named these *Thelohania diazoma* and *Nosema necatrix*, respectively, believing them to constitute a dual infection. Subsequently, however, Fowler & Reeves (3) obtained evidence that these spores were produced by a single microsporidan species for which they proposed retention of the name *N. necatrix* Kramer; the name *T. diazoma* was to be suppressed. Evidence for the dimorphic nature of this organism was also found by Maddox (8). Recently, Pilley (9) proposed *N. necatrix* as the type species of a new dimorphic genus, *Vairimorpha*.

Burenella differs from *Vairimorpha* in hosts, tissue specificity, and ultrastructure. The NMB spores of *Burenella* develop in the hypodermis, and MB spores in the fat body of ants, while both spore types of *Vairimorpha* develop side by side in the fat body of the lepidopterous hosts. The pansporoblast membrane of *Burenella* is not persistent (it is seen only with the aid of electron microscopy) while that of *V. necatrix* is so highly persistent that it is difficult to disrupt the pansporoblast to obtain spore measurements (6). The polar filaments of both spores of *Vairimorpha* are arranged in single coils at the periphery of the



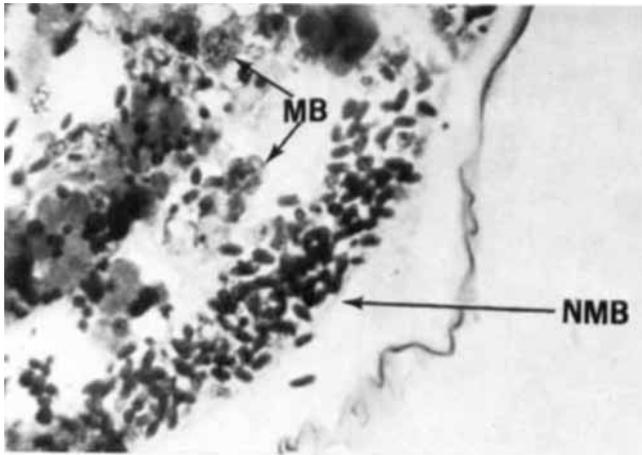


Fig. 6. Nonmembrane-bounded (NMB) and immature membrane-bounded (MB) spores in the hypodermis and adipose tissue, respectively.

spores, and the polaroplasts are loosely lamellate. The polar filaments of *Burenella* are coiled irregularly and the polaroplasts are indistinct.

The generic name of this microsporidium was chosen in recognition of Dr. William F. Buren, of the University of Florida, who made the original observations that resulted in the discovery of microsporidan infections in ants. Also, his contributions to the taxonomy of *Solenopsis* and to myrmecology in general will be of great value to pathologists interested in ants. The type species is *B. dimorpha* by monotypy.

In the species *Burenella dimorpha* sp. n., the living NMB spores are coniform and faint yellow amber in color. They stain well with Giemsa and Heidenhain's hematoxylin. The polar filament, with ~ 26 coils, is extruded easily under manual pressure and tends to form a tangled mass at the point of extrusion.

Living MB spores are broadly oval and pronouncedly yellow amber color. They stain very poorly with Giemsa and Heidenhain's hematoxylin. The pansporoblast membrane is extremely fragile and bursts upon dissection from the host. Consequently, mature MB spores are not seen, and ooctets of immature spores are seen only rarely. The length of the polar filament is striking, having ~ 57 coils. It is extruded with difficulty under manual pressure in a manner similar to that of the NMB spore.

Burenella dimorpha appears to have 2 sequences of merogony. The first involves uninucleate cells with deeply staining cytoplasm (Giemsa) and compact nuclei that become binucleate and divide. The 2nd sequence involves binucleate cells with moderately staining cytoplasm and less dense nuclei, that become tetranucleate and divide to produce 2 binucleate cells (Fig. 7).

Two sequences of sporogony follow merogony. One sequence is similar to sporogony in the genus *Nosema*, producing NMB spores, the other sequence is similar to sporogony in the genus *Thelohania*, producing MB spores in groups of eight.

Development of NMB spores precedes that of MB spores, and the former are more numerous. Typically, mature NMB spores

are abundant, and MB spores are absent in pupae with minimal symptoms. In pupae with severe symptoms, MB spores usually constitute ~ 25-40% of all spores. In a few colonies, however, that had very high infection rates, MB spores were found only in the most advanced infections and constituted 2% or fewer of all the spores present in these individuals.

The pathologic manifestations caused by *B. dimorpha* infection are quite characteristic. They are observed only in pupae, and are the result of infection of the hypodermis. A clear area in the occipital region of the head, which appears about the time the developing eyes become prominent, is usually the first noticeable change. Later, similar clear areas appear in the petiole and gaster, and the eyes become irregular in outline and appear sunken (Fig. 8). Pupae having such changes do not mature or even melanize. Instead, the clear areas increase in size, and the cuticle eventually ruptures. Infected pupae that have ruptured have been observed in laboratory colonies, and it seems probable that infection is transmitted to larvae by contamination from such pupae, and by worker ants tending the brood. Infected adults or infected melanized pupae have never been observed, and we believe the infection is almost invariably fatal.

We have experimentally transmitted *B. dimorpha per os* to healthy colonies of *S. geminata*, *S. invicta*, *S. richteri*, and *S. xyloni*. The course of the disease is identical in all 4 species. Infection rates of ~ 50% were achieved in the first series of tests. We have since routinely transmitted *B. dimorpha* in *S. geminata* and *S. invicta* in the laboratory and have often obtained infection rates of 100% within colonies. On a few occasions, infection rates of 100% have been observed in naturally infected field-collected colonies of *S. geminata*; however, the rate within these colonies is usually less than 5%.

We attempted to demonstrate dimorphism in this microsporidan by separating the spore types for feeding tests by density gradient centrifugation but were unable to achieve satisfactory separation. We have now examined over 50 colonies (12 of these from 7 localities other than the type locality) and have always observed both spore types in all ants with advanced pathologic manifestations. Dimorphism is known to occur in *Amblyospora* and *Parathelohania* (Thelohaniidae) (4). In these genera MB spores are not infective *per os*. Since sporogony in *Burenella* appears similar to sporogony in these genera, infection transmitted *per os* in our tests is probably due only to the NMB spores.

Burenellidae fam. n.

We believe the genera *Burenella* and *Vairimorpha* are related and that they differ significantly from other genera. Therefore we propose a new family for species having 2 sporogonic sequences, one producing NMB spores from disporous sporonts, and the other producing MB spores from octonucleate sporonts. The latter are enclosed in a pansporoblastic membrane that may or may not be persistent.

Certain genera of the family Thelohaniidae Hazard & Oldacre (4) also produce NMB spores in addition to MB spores. The NMB spores of Burenellidae, however, arise from disporous sporonts, while a variable number (6-40) of NMB spores arise from plasmodia in Thelohaniidae. Also, the MB sporonts of species of the Burenellidae do not secrete granules as do those

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Figs. 2-5. [Electronmicrographs of spores: N, nuclei; P, polaroplast; PF, polar filament; PM, pansporoblast membrane.] 2, 5. Sections through membrane-bounded spores. × 33,000. 3. Sporont of nonmembrane-bounded spores with nuclei in a diplokaryon arrangement. × 7,000. 4. Sporonts of a membrane-bounded spore in a pansporoblast membrane. × 7,000.

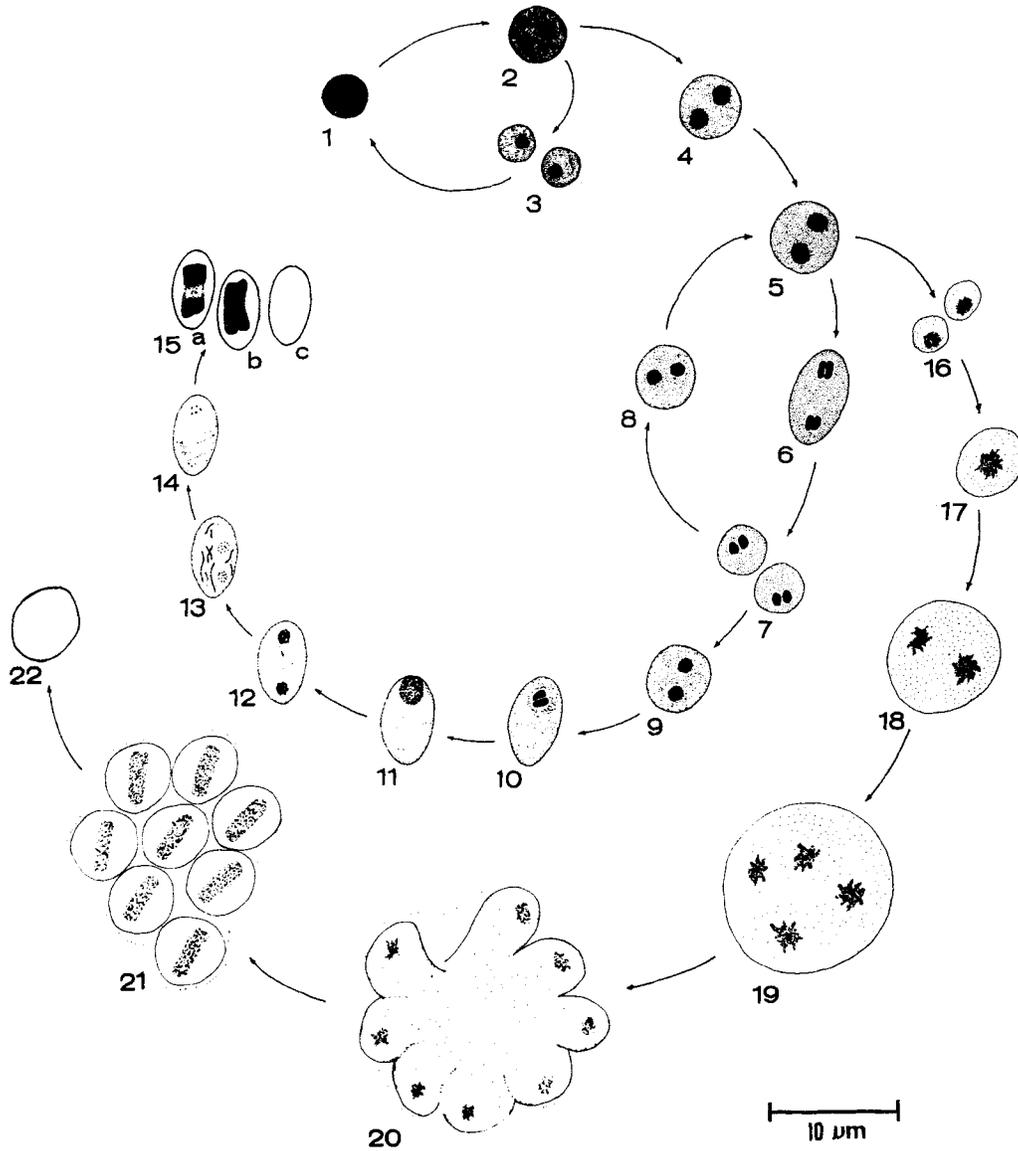


Fig. 7. Diagram of the life cycle of *Burenella dimorpha* gen. n., sp. n. Giemsa stain. First merogony (1-3); transitional stage between 1st and 2nd merogony (4); 2nd merogony (5-8); 1st sporogony (9-13); immature nonmembrane-bounded spore (14); stained nonmembrane-bounded spores with different staining intensities (15a, b); unstained nonmembrane-bounded spore (15c); 2nd sporogony (16-20); unstained immature membrane-bounded spores (21); mature MB spore (22).

of the Thelohaniidae (4), but do develop tubules within the pansporoblastic membrane during sporulation.

TAXONOMIC SUMMARY

Burenella gen. n.

Diagnosis.—Disporous sporonts sporulate in hypodermis producing binucleate, non-membrane bounded (NMB) spores; multinucleate sporonts produce 8 uninucleate, membrane bounded (MB) spores in fat cells; pansporoblastic membrane not persistent in the single known species; NMB spores coniform, MB spores broadly oval; polar filament of both spores nearly uniform in diameter, and, in the single known species, longer in the MB spores; polaroplast structure indistinct, exospores thin, endospores thick; surfaces smooth in both spores.

Burenella dimorpha sp. n.

Diagnosis (measurements in μm).—With characteristics of the genus; NMB spores coniform, faint yellow amber in color, measuring 6.4 ($6-7$) \times 2.9 ($2.5-3$), stain well with Giemsa and Heidenhain's hematoxylin; MB spores broadly oval, pronouncedly yellow amber in color, measuring 6.1 ($6-6.5$) \times 4.2 ($4-4.5$), stain poorly with Giemsa and Heidenhain's hematoxylin; pansporoblast membrane extremely fragile, MB spores rarely seen in octets by light microscopy; polar filament of NMB spore with 26 coils, MB spore with 57 coils; NMB spores predominate in number.

Type host.—Immature stages of the tropical fire ant, *Solenopsis geminata* (Fabricius). State Road 26 East of Interstate Highway 75, Gainesville, Alachua Co., Florida. Holotype: USNM No. 24511; Paratypes: USNM No. 24512.

Infection sites.—NMB spore in hypodermis; MB spore in fat cells.

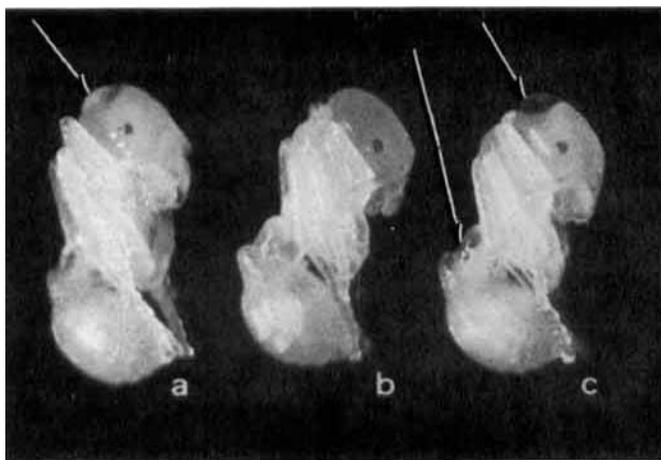


Fig. 8. Pathologic changes in pupae (a, c) infected with *B. dimorpha*. Note the clear areas in the occipital region of the head and the petiole (arrows) and the irregular outlines of the eyes. Compare the appearance of the infected pupae with that of an uninfected pupa (b). $\times 15$.

BURENELLIDAE, fam. n.

Diagnosis.—Two sporogonic sequences: one producing NMB spores from disporous sporonts, the other producing MB spores from octonucleate sporonts; pansporoblast membrane of latter may or may not be persistent; microtubules but not granules develop within pansporoblast space during sporulation.

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BOOK REVIEW . . .

Grun, P. 1976. *Cytoplasmic Genetics and Evolution*. Columbia University Press, 562 W. 113th St., New York, NY 10025. xi + 425 pp. hardback, \$25.00.

"This book," in the author's words, "is about organisms as multiple genetic combinations and the effects of their interactions on heredity and evolution." In those terms, especially as regards heredity, the book is very successful. Grun surveys mitochondria, plastids, symbiotes, and other nonchromosomal factors in a lucid, thorough, and generally thoughtful manner. There are well over 1000 references ranging from viruses to prokaryotes and eukaryotes, including extensive treatment of higher plants and animals. Perhaps the most impressive aspect of this attractive volume is the even-handed analysis provided to taxonomically disparate groups. The reader is not aware that Grun's research specialty lies with cytoplasmic sterility in potatoes.

This book can be recommended to anyone familiar with basic genetics as an authoritative, readable and interesting overview of cytoplasmic inheritance. Specialists already active in this area

will be much aided by the range of Grun's treatment mentioned above.

Where the book is lacking is in the area of evolution. This is not the author's fault; it is inherent in the subject matter. The study of nonchromosomal inheritance inevitably developed as a complement to chromosomal studies. Its unique emphasis, if it has one, is usually epigenetic—how chromosomal and non-chromosomal factors interact to produce a given phenotype. Hence students of nonchromosomal factors (cytoplasmic genetics) have spent little time with evolutionary problems. Just as developmental biology itself has achieved no great evolutionary generalizations beyond the biogenetic law and the inevitable comparisons seen in vertebrate embryos, nonchromosomal factors must be broadly involved in evolution but research excitement over their role is hard to come by. Some years from now that assessment may be totally different. If so, Grun's book may well appear as a pioneering review which helped revise that assessment.—EARL D. HANSON, *Department of Biology, Wesleyan University, Middletown, CT 06457.*