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Vairimorpha invictae N. Sp. (Microspora: Microsporida), a Parasite of the Red Imported Fire Ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae)[1,2]

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ABSTRACT. *Vairimorpha invictae* n. sp. infects the red imported fire ant, *Solenopsis invicta* Buren, in Brazil. The parasite is dimorphic, producing two morphologically distinct types of spores, which develop sequentially in the same fat cells or oenocytes in the fat body. The binucleate free spores develop from disporous sporonts; the uninucleate octospores develop from multinucleate sporonts within a sporophorous vesicle. Infected cells are transformed into large sacs which contain both types of spores in mature adult hosts. Mature free spores are often present by the time the larvae pupate, but mature octospores are found only in adult hosts. Masses of spores may be seen through the intact cuticle by low power phase-contrast microscopy; there are no other physical signs and no behavioral signs of infection. Attempts to transmit the infection in the laboratory failed.

THE red and black imported fire ants, *Solenopsis invicta* Buren and *Solenopsis richteri* Forel, were introduced into the United States from South America ca. 1940 and 1920, respectively. *Solenopsis invicta* has become the dominant species and now infests ca. 9.3×10^7 ha (2.3×10^8 acres) in nine southeastern states. Should either of these species be transported by man to the more humid or irrigated areas of the Southwest, their range could greatly increase. Efforts to control these medical and agricultural pests with toxicants have been the subject of serious controversy for many years (9). The United States Department of Agriculture has, therefore, entered into a cooperative agreement with the Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA, the Brazilian agricultural research organization) to search for natural enemies of these ants in their native lands. The goal of this project is to introduce into the United States a complex of specific natural enemies which will exert some degree of control or stress on our fire ant populations.

Three species of protozoa that parasitize fire ants have been described. *Burenella dimorpha* Jouvenaz & Hazard (Microsporida) (4) and *Mattesia geminata* Jouvenaz & Anthony (Neogregarinida) (5) infect a nearctic fire ant, *Solenopsis geminata* (Fabricius); the third protozoon, *Thelohania solenopsae* Knell, Allen & Hazard (Microsporida) (7) infects *S. invicta* in Brazil. We describe here a second microsporidian parasite of *S. invicta*, for which we propose the name *Vairimorpha invictae*.

MATERIALS AND METHODS

One infected colony of *S. invicta* (including the queen) was collected in the State of Mato Grosso, Brazil and hand-carried (under USDA and Brazilian permits) to our laboratory in Florida. The identity of the colony was confirmed by Drs. D. P. Wojcik, USDA, Gainesville, and J. C. Trager, University of Florida. This colony and healthy colonies of *S. invicta* from Florida were maintained as described by Banks et al. (1).

Spore suspensions. Spore suspensions were prepared by macerating ants en masse in distilled water with a glass tissue grinder and partially purifying the crude extract by Percoll® (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation (6). Spore suspensions were stored at 4°C in distilled water.

Transmission tests. Transmission tests were conducted by feeding a small, healthy colony of *S. invicta* (~10,000 workers) with boiled egg yolk wetted with spore suspension daily for three days. This technique is routinely used for propagating *B. dimorpha* in our laboratory. In addition, brood from the infected colony was transferred to a healthy colony of *S. invicta* (fire ants readily adopt conspecific immatures).

Light microscopy. Diagnosis of infection was made by examining fresh smears of whole specimens by phase-contrast microscopy or by examining whole living adult ants by phase-contrast microscopy at 100–200× for internal spore masses. Giemsa-stained smears were prepared by air-drying smears, fixing in methanol for 5 min, staining with 10% (v/v) stock Giemsa in phosphate-buffered (pH 7.4) distilled water for 10 min, and rinsing in distilled water adjusted to ~pH 6.8 with acetic acid. Fresh spores immobilized between a layer of 1.5% Noble Agar® (Difco Laboratories, Detroit, MI) and a coverslip were measured with a calibrated A. E. I. Cook image-splitting micrometer.

Transmission electron microscopy. Tissue specimens were prefixed in buffered 1% (w/v) OsO_4 (0.1 M sodium cacodylate buffer, pH 7.5) for 30–60 min at room temperature, rinsed with buffer, and fixed in 2.5% (v/v) glutaraldehyde–1% (v/v) acrolein (same buffer). Specimens were washed in buffer and usually stored in Histocon® (Polysciences, Inc., Warrington, PA) in the refrigerator overnight to several days prior to postfixation. Specimens were washed in buffer, postfixed in buffered 1% OsO_4 for 2 h at room temperature, washed in deionized water, and stained en bloc in 0.5% (w/v) aqueous uranyl acetate overnight. Specimens were dehydrated with acidified 2,2-dimethoxypropane (8) and infiltrated and embedded in Spurr-Quetol 651 resin (13). Spore pellets were similarly prepared, with and without 5% sucrose in the buffer.

Gold sections, cut with an LKB ultramicrotome, were poststained with 2% aqueous uranyl acetate followed by lead citrate (12). Grids were examined and photographed at an accelerating voltage of 75 kV in a Hitachi H-600 electron microscope.

DESCRIPTIVE ACCOUNT AND DISCUSSION

Morphology and life cycle. Meronts of the first generation (Figs. 1–4) are small cells (~4.5–5 μm) with cytoplasm that stains lightly to moderately and rounded, compact nuclei that stain intensely (Giemsa). The majority (>95%) are binucleate; most of the remainder appear to be uninucleate, but a few cells are tetranucleate. Meronts of the second generation (Figs. 5–8) are larger cells (~10–11 μm) with vacuolated or irregularly staining cytoplasm and larger, irregular and less compact nuclei. These cells are binucleate or tetranucleate with the nuclei in diplokaryotic arrangement. Their transformation into sporonts was easily followed by examining numerous cells, thus establishing the sequence of generations.

Two sequences of sporogony occur. One sequence produces binucleate "free spores" (spores do not develop within a spo-

[1] The authors wish to express their appreciation to Mrs. Susan W. Avery, USDA, Gainesville, Florida, for assistance with electron microscopy and to Mr. Paul M. Choate, University of Florida, for photographic darkroom work. Drs. Daniel P. Wojcik, USDA, Gainesville, and James C. Trager, University of Florida, identified *S. invicta*.

[2] Mention of a proprietary product does not constitute endorsement by the USDA.

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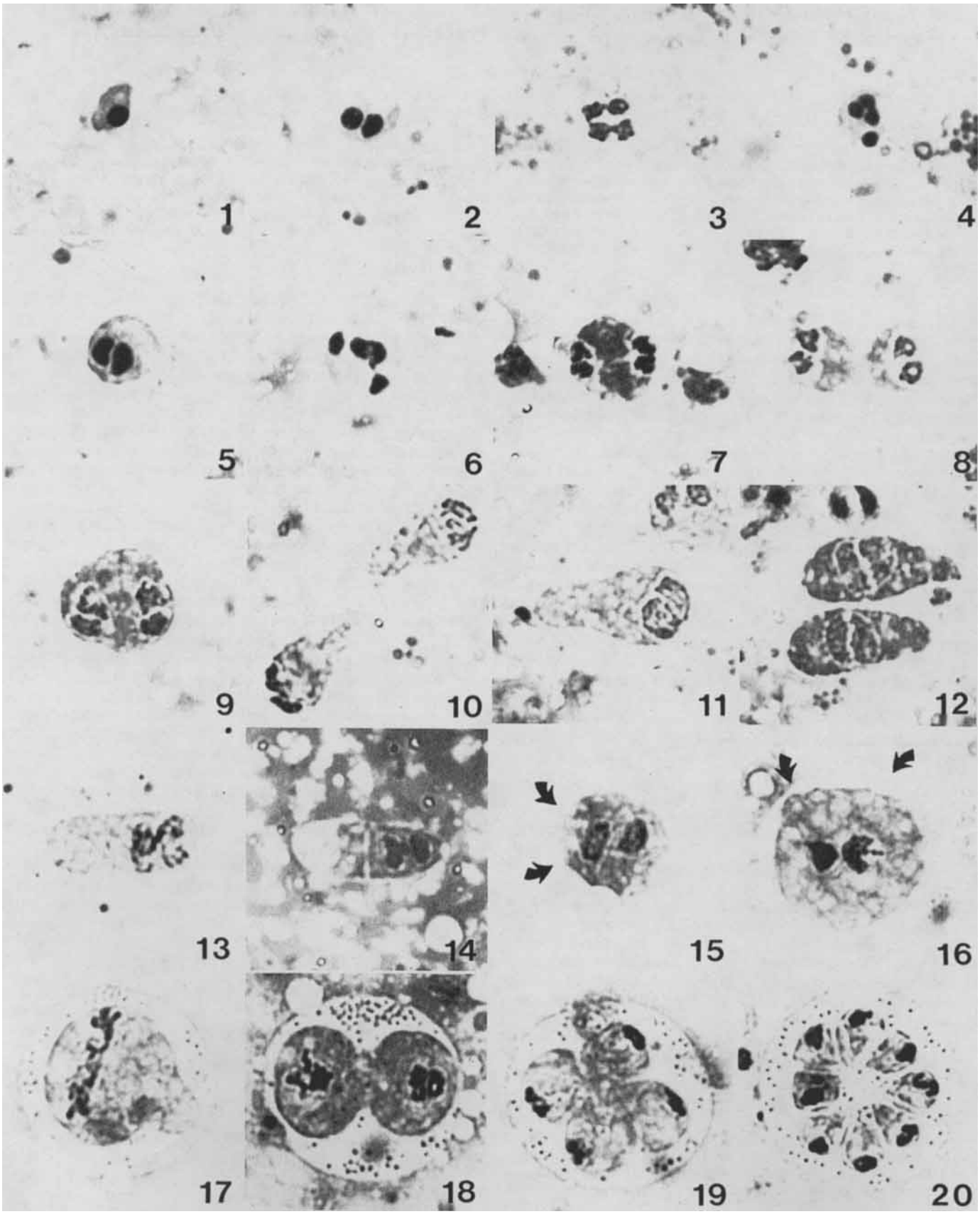
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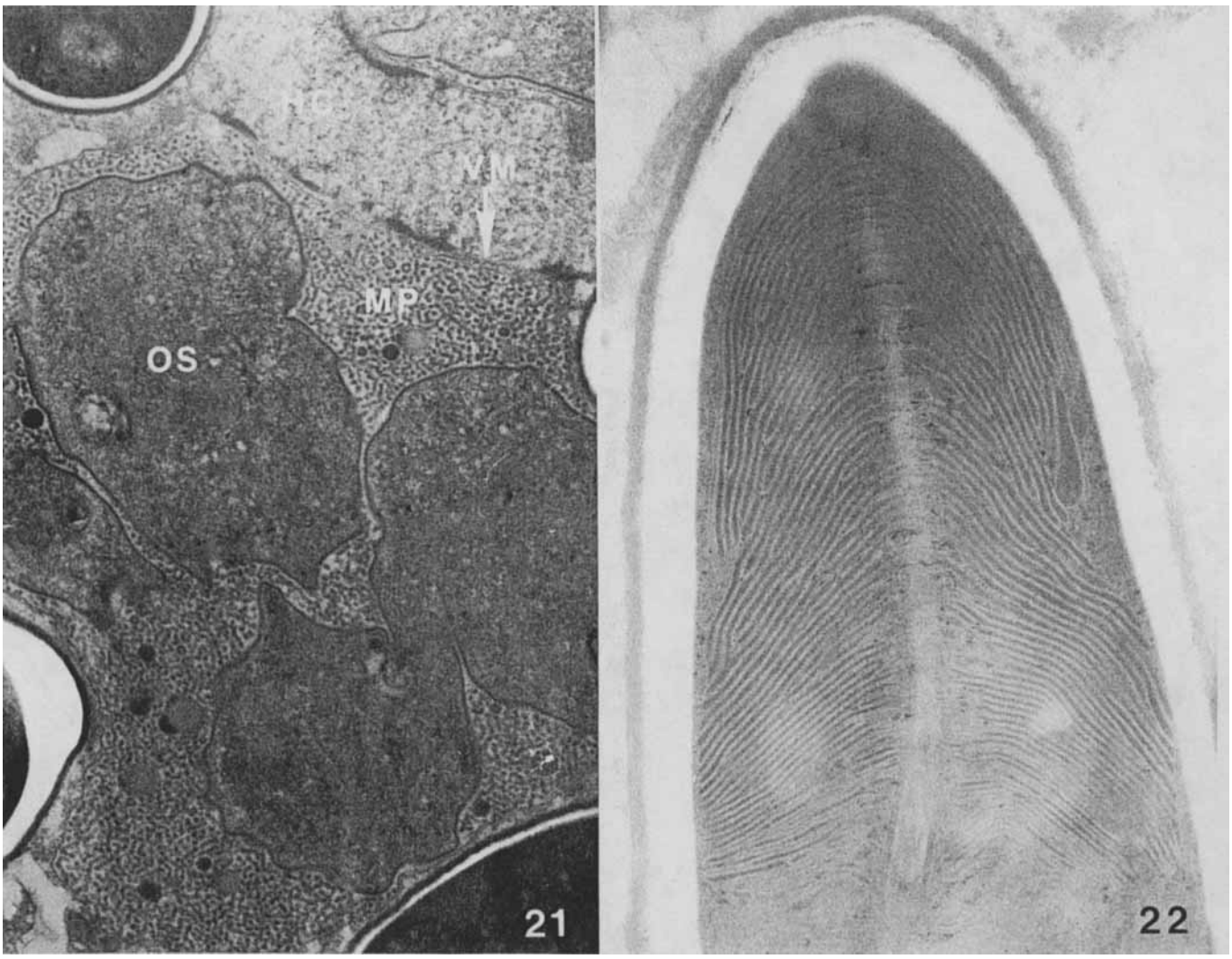

Figs. 21, 22. Electron micrographs of *Vairimorpha invictae*. **21.** Octosporoblasts in sporophorous vesicle. ×15,000. Note granular and tubular metabolic products in lumen and electron-dense material deposited on the vesicle membrane. HC, host cytoplasm; VM, vesicle membrane; MP, metabolic products; OS, octosporoblast. **22.** Lamellate polaroplast of free spore. ×50,000.

rophorous vesicle) from disporous sporonts (Figs. 9–14). The second sequence produces uninucleate "octospores" (spores develop in octets within a sporophorous vesicle). The octosporonts enlarge to the approximate size of the future sporophorous vesicle (26–30 μm, Giemsa stain), then undergo two nuclear divisions and cytokinesis within the lumen of the vesicle (Figs. 15–20). The octosporont in Fig. 17 appears to be in metaphase I as described by Hazard & Brookbank (2) for an *Amblyospora* sp.

The lumen of the sporophorous vesicle contains granular and tubular metabolic products during sporogenesis (Fig. 21). The convoluted layers of metabolic products shown by Malone & Canning (10) for *Vairimorpha plodiae* and Jouvenaz & Hazard (4) for *B. dimorpha* have not been seen in *V. invictae*. We doubt the taxonomic significance—especially above the species level—of these dynamic accumulations of metabolic products. Note also in Fig. 21 the deposition of electron-dense material on the sporophorous vesicle membrane. The sporophorous vesicle of *V. invictae* is strong and persistent; that of *B. dimorpha*, in which thickening does not occur, is so delicate that it ruptures upon dissection of the host. Sections of four octosporoblasts with early spore wall formation (thickening of the cell membrane) are visible in this electron micrograph (Fig. 21).

The binucleate free spores are bacilliform (Fig. 25) and measure 11.2 ± 3.4 by 3.1 ± 0.3 μm (n = 20). In phase-contrast microscopy, mature free spores are refractile and light amber

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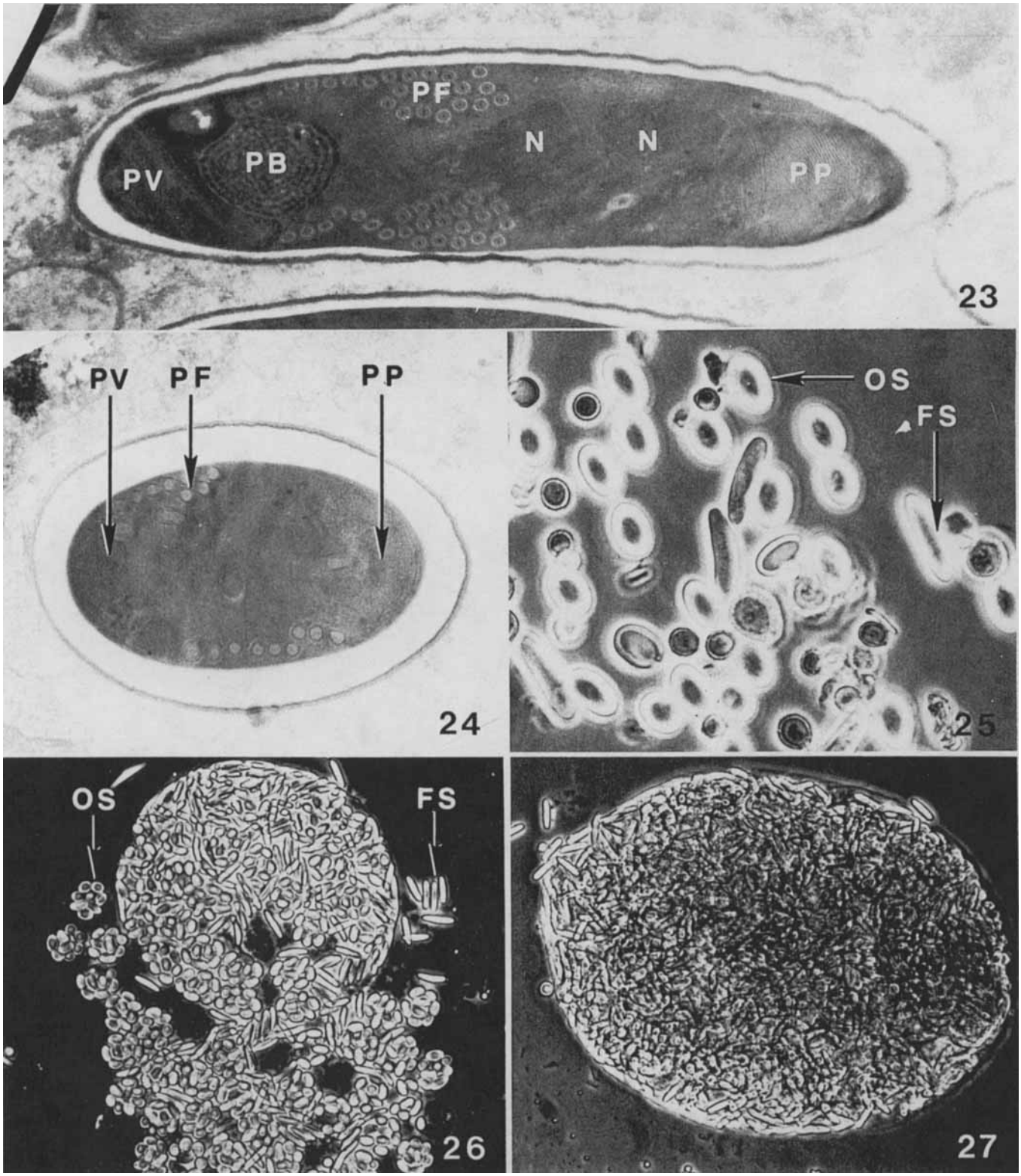
Figs. 1–20. Photomicrographs of vegetative stages of *Vairimorpha invictae* (methanol-fixed, Giemsa stain). ×1450. **1–4.** Uninucleate (uni- and bidiplokaryotic?) and tetranucleate first generation meronts. **5–8.** Larger second generation meronts. Note the deeper staining, vacuolated cytoplasm and ring nuclei in Figs. 7, 8. **9–14.** Free spore sporogony. The cell in Fig. 15 is within a small faint sporophorous vesicle (arrows). Note the sporophorous vesicle (arrows) in Fig. 16.



Figs. 23–27. Spores of *Vairimorpha invictae*. 23. Transmission electron micrograph of free spore. ×20,000. 24. Transmission electron micrograph of octospore. ×15,000. 25. Photomicrograph of living spores. ×1500. 26, 27. Photomicrographs of enlarged infected cells containing spores of both types. ×500. Note the octospores in octets. FS, free spores; OS, octospores; N, nucleus; PB, posterior body; PF, polar filament; PP, polaroplast; PV, posterior vacuole.

internally. The surface of the spore is smooth as seen by scanning electron microscopy. The polar filament is isofilar with 24–26 coils. The polaroplast is loosely lamellate (Fig. 22), and a posterior vacuole and posterior body are present (Fig. 23).

The uninucleate octospores are ovoid and very slightly narrower at the anterior pole (Figs. 24, 25) and measure 6.3 ± 0.25 by 4.2 ± 0.7 μm (n = 20). In phase-contrast microscopy, mature octospores are refractile and deeply amber internally. The surface of the octospore is also smooth. The polar filament is isofilar and has ~9 coils. The polaroplast is lamellate, and a posterior vacuole and posterior body are present (Fig. 24). Due to the strength of the sporophorous vesicle membrane, octospores are seen in octets in fresh preparations (Fig. 26).

Tissue specificity. Vairimorpha invictae develops in cells in the head, petiole, and gaster. We are unable to determine to our satisfaction whether these are fat cells or oenocytes within the fat body. Parasitized cells are transformed into large sacs up to ~0.2 mm in diameter (Figs. 26, 27) which may be seen as masses of spores within the intact host at 100–200× magnification. The spore sac in Fig. 27 is 0.17 mm in length. Both types of spores develop in the same cells, but sequentially. Mature free spores are present by the time of pupation or very soon thereafter. Octospore development is delayed until late in pupal life; mature octospores are not often seen even in callow adults. Octospores constituted 53.4% of the spores in an aqueous extract of 200 adult ants from the laboratory colony.

Transmission and signs. There are no physical or behavioral signs of infection other than the spore masses which may be seen through the intact cuticle by phase-contrast microscopy. Our attempts to transmit infection perorally and by placing brood from the diseased colony in a healthy colony were unsuccessful. The intracolonial prevalence of infection in the laboratory colony was 71%. In Brazil, ~2% (14 of 683 colonies studied) were infected.

Classification. The family Burenellidae was created by Jouvenaz & Hazard (4) for dimorphic microsporidia having disporous and octosporous sporogony. Spore dimorphism also occurs in certain genera of the family Thelohaniidae Hazard & Oldacre, 1975 (3); however, the free spores of these species arise from plasmodia which have 8–40 nuclei (2). Two genera, *Burenella* (monotypic) (4) and *Vairimorpha* (11), differentiated by host orders, tissue specificities, and certain ultrastructural characters, were assigned to Burenellidae (4). It now appears to us that this classification is in need of further study. Viewed holistically, the two genera appear to be quite different; however, it is the sum of a number of quite different species characters that separates them. We assign the microsporidium described here to *Vairimorpha* because both sporulation sequences occur in the same tissue. In addition, the sum of specific characters agrees more closely with that of *Vairimorpha.*

TAXONOMIC SUMMARY

Vairimorpha invictae n. sp.

Diagnosis. Two morphologically distinct types of spores in adult host. Binucleate free spores bacilliform, 3.1 ± 0.3 × 11.2 ± 3.4 μm. Spore surface smooth; endospore thin, ~0.1 μm; polar filament isofilar, 24–26 coils. Uninucleate octospore ovoid, 4.2 ± 0.7 × 6.3 ± 0.2 μm. Spore surface smooth; endospore thick, ~0.5 μm; polar filament isofilar, ~9 coils. Sporophorous vesicle membrane persistent. Both spore types together in membrane-bounded sacs up to ~0.2 mm diameter in adult host. Mature free spores present in late larvae or early pupae; mature octospores in adult hosts only.

Host. Red imported fire ant, *Solenopsis invicta* Buren, 1972.

Infection site. Fat cells or oenocytes in the fat body of the head, petiole, and gaster.

Holotype. Cuiaba, Mato Grosso, Brazil. Museu Zoológico, Universidade de São Paulo, SP, Brazil; with host collection.

Paratypes. Locality as for holotype. Museu Zoológico, Universidade de São Paulo, SP, Brazil and USNM no. 34480.

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