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Johenrea locustae n.g., n.sp. (Microspora: Glugeidae): A Pathogen of Migratory Locusts (Orthoptera: Acrididae: Oedipodinae) from Madagascar

CARLOS E. LANGE,*,‡ JAMES J. BECNEL,§ ELÉONORE RAZAFINDRATIANA,‖ JOHN PRZYBYSZEWSKI,† AND HANTA RAZAFINDRAFARA‖

**CIC-CEPAVE, Universidad de La Plata, Calle 2 N° 584, 1900 La Plata, Argentina; ‡Entomology Research Lab, Montana State University, Bozeman, Montana 59717; §USDA-ARS, Medical and Veterinary Entomology Research Lab, P.O. Box 14565, Gainesville, Florida 32604; and ‖Direction de Protection Végétal and Deutsche Gesellschaft für Technische Zusammenarbeit, Antananarivo, Madagascar*

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A new microsporidium was isolated from the migratory locust, *Locusta migratoria capito,* collected in Madagascar. This new species was found to be haplokaryotic throughout development, produced polysporophorous vesicles of parasite origin, and there was conspicuous xenoma formation. The xenoma was a complex structure composed primarily of fat body cells. The wall of the xenoma was bound by a basement membrane beneath which was a region of collagen-like fibers. Multiplication of the parasite occurred by way of both a schizogonic phase and by a sporulation phase. Schizonts divided either directly or with the intervention of paucinucleate plasmodia into additional schizonts. At some point, plasmodia developed (with an increase in size and number of nuclei) into sporogonial plasmodia with typically 16 nuclei. Sporogonial plasmodia elaborated an interfacial envelope within which they underwent sporogony. Sporophorous vesicles normally contained 16 spores but also 8 and rarely 32 spores were formed. Spores were elongate ovoid (sometimes slightly curved) and measured 8.3 by 3.8 μm (fresh) and 6.9 by 4.2 μm (fixed). Based on the development of the microsporidium and the features of the xenoparasitic complex, a new species and genus *Johenrea locustae* n.g., n.sp. is proposed.

KEY WORDS: *Johenrea locustae; Locusta migratoria;* microspora; taxonomy; biological control; locusts; xenoma; Madagascar.

INTRODUCTION

The migratory locust, *Locusta migratoria,* is widely distributed throughout the whole of the temperate and tropical parts of the eastern hemisphere and causes severe agricultural damage over extensive areas (COPR, 1982). The subspecies *Locusta migratoria capito* inhabits Madagascar and is regarded as the most important agricultural pest of the island (Steedman, 1990). Control of this pest relies primarily on the application of chemical products. In the hope of finding alternatives to chemical control, a survey for pathogens associated with the migratory locust and other acridiomorphs of Madagascar was initiated in 1993. One outcome of this exploration has been the discovery of a microsporidium new to science. This report describes the morphological and developmental features of this new microsporidium as well as providing information on transmission and host specificity. A new genus for this species is proposed and its affinities to other microsporidia are discussed.

MATERIALS AND METHODS

Six species of grasshoppers and locusts (nymphs and adults) were collected during April–May 1993–1994 from 18 locations in southern Madagascar and identified as *L. m. capito, Acrotylus patruelis, Aiolopus rodericensis, Chromacrida radamae, Cyrtacanthacris tatarica,* and *Oedaleus virgula.* Five locations were in the vicinity of Amboasary in the southeast; the remaining locations were within a 100-km radius of Tulear in the southwest part of the island. Insects were maintained in wire-screened cages in the laboratory until examination.

For pathogen detection, approximately 30–50 individuals of each species per location were either homogenized whole in tissue grinders or dissected in order to remove samples of organs and tissues. Fresh mounts of homogenates and tissue/organ samples were examined with a phase-contrast microscope.

The new microsporidium was found in solitary phase adults of *L. m. capito* collected in early May 1994 near Ambalavenoka, about 50 km north of Tulear. Spore suspensions were prepared from infected individuals

† Deceased.

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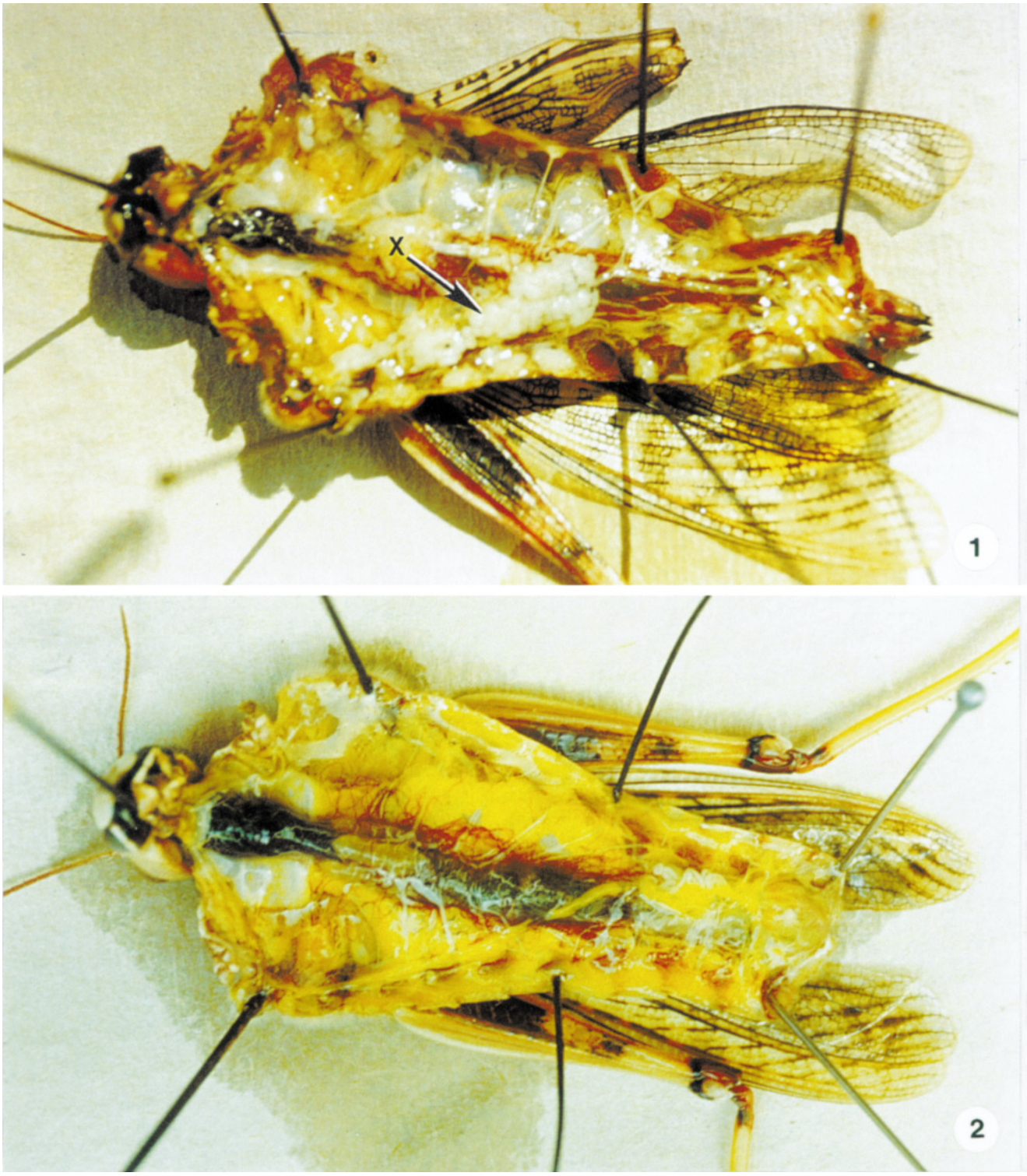
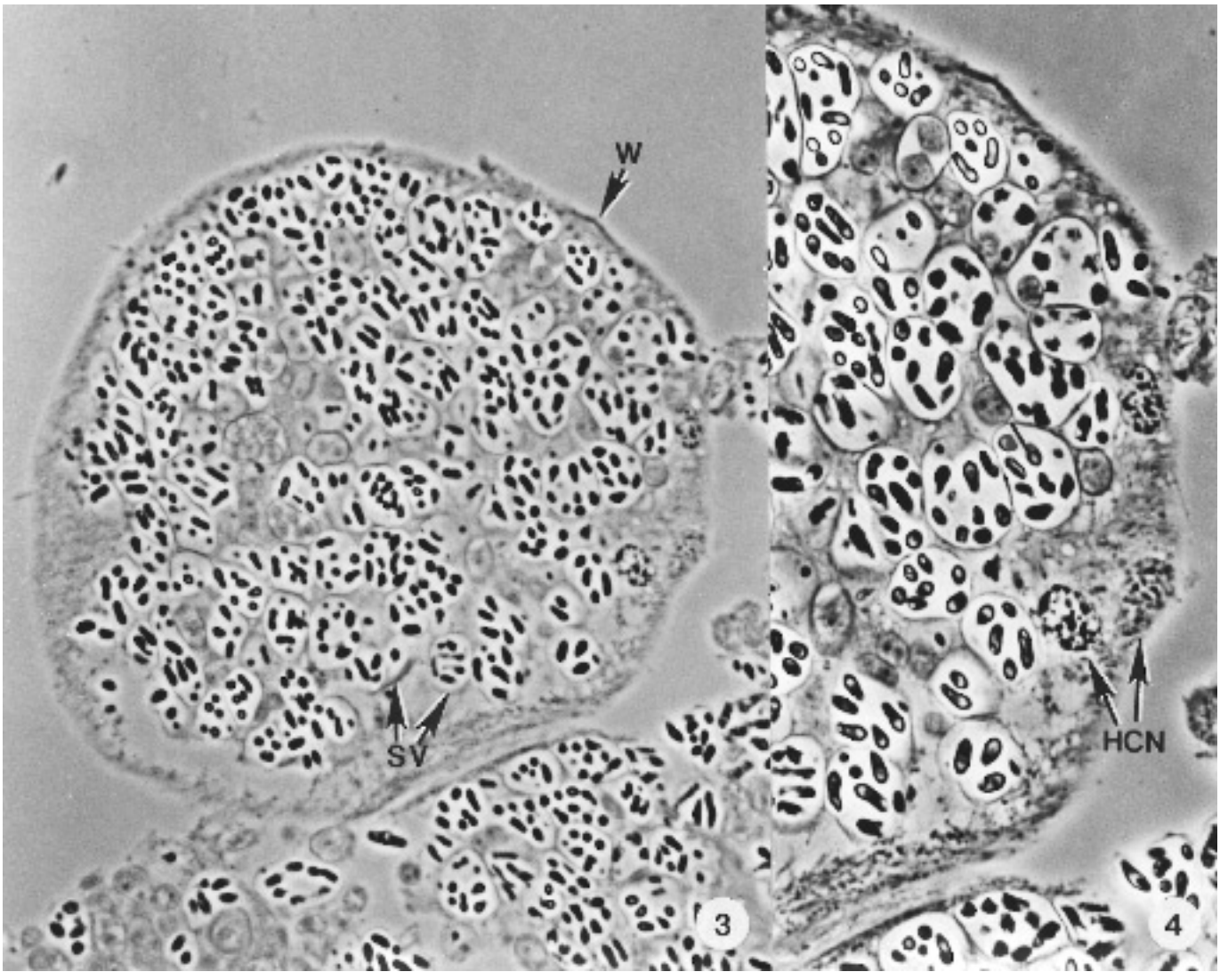


FIGS. 1 and 2. Gross pathology of infections by *Johenrea locustae* in *Locusta migratoria migratorioides*. (1) Ventrally dissected infected locust showing xenoma (X) formation. ×2.5. (2) Ventrally dissected healthy locust showing normal development of fat body (yellow tissue, ×2.7).

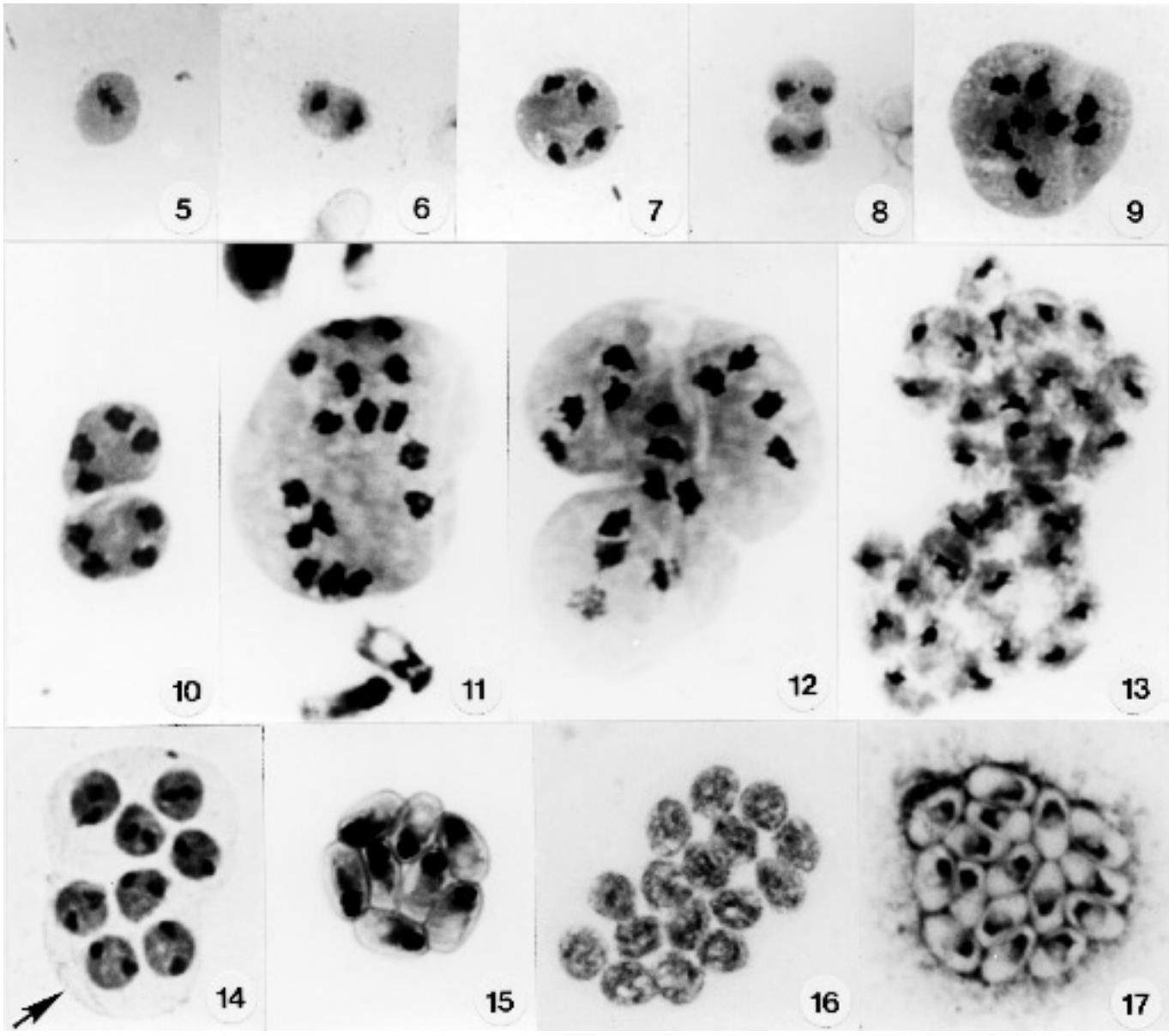


FIGS. 3 and 4. Xenoma of *J. locustae* in *L. m. migratorioides.* Thick sections under phase-contrast microscopy. (3) Rounded xenoma showing the well-defined wall (W), numerous sporophorous vesicles (SV), and some stages in schizogony and sporogony. ×300. (4) Higher magnification of part of the xenoma revealing host nuclei (HCN). ×400.

following the homogenization procedure used by Henry *et al.* (1973). Laboratory-reared third instar nymphs of *L. m. capito* and *Locusta migratoria migratorioides* were challenged with spore suspensions according to the protocols of Henry (1985a). The concentration of spores administered per nymph was not measured. Nymphs of two grasshopper species in the subfamily Melanoplinae, one from North America, *Melanoplus sanguinipes,* and the other from South America, *Baeacris punctulatus,* were also challenged. Inoculated locusts and grasshoppers were maintained as described by Henry (1985b) until they were sacrificed and examined with Giemsa-stained smears for infection and under phase microscopy for the presence of spores.

Two different types of preparations were used for light microscopy studies. Fresh mounts with distilled water or one-quarter-strength Ringer's solution (Poinar and Thomas, 1984) were examined with phase-contrast microscopy to visualize membranes around stages; the total number of nuclei per stage, however, was difficult to assess with accuracy due to varying planes of focus. Giemsa-stained permanent preparations (Wang *et al.,* 1991) were used to determine the number of nuclei per stage but membranes were extremely difficult to resolve. Fresh and fixed spores were measured with an ocular micrometer.

For transmission electron microscopy, infected tissues were fixed for 1 hr at 4°C in 2.5% (v/v) glutaraldehyde buffered with 0.1 M cacodylate buffer (pH 7.4), postfixed in 1% aqueous OsO_4 (w/v), and *en bloc* stained with 1% uranyl acetate. Dehydration was through an ascending acetone series after which samples were embedded in Spurr's resin. Thick (1 μm) sections observed under phase-contrast microscopy



FIGS. 5–17. Developmental stages and spores of *J. locustae* from Giemsa-stained smears. ×2000. (5) Uninucleate stage undergoing karyokinesis. (6) Binucleate stage. (7) Tetranucleate plasmodium. (8) Plasmotomy of tetranucleate stage. (9) Octonucleate plasmodium. (10) Plasmotomy of octonucleate plasmodium. (11) Plasmodium with 16 nuclei. (12) Plasmotomy of plasmodium with 16 nuclei. (13) Final division of sporogonial plasmodium with 16 nuclei, producing sporoblasts. (14) Vesicle containing eight rounded bodies (probably sporoblast mother cells) with dividing nuclei. Note the envelope of the sporophorous vesicle (arrow). (15) Eight spores in a sporophorous vesicle which is not evident. (16) Vesicle with 16 sporoblasts. (17) Sixteen mature spores in a sporophorous vesicle which is not evident.

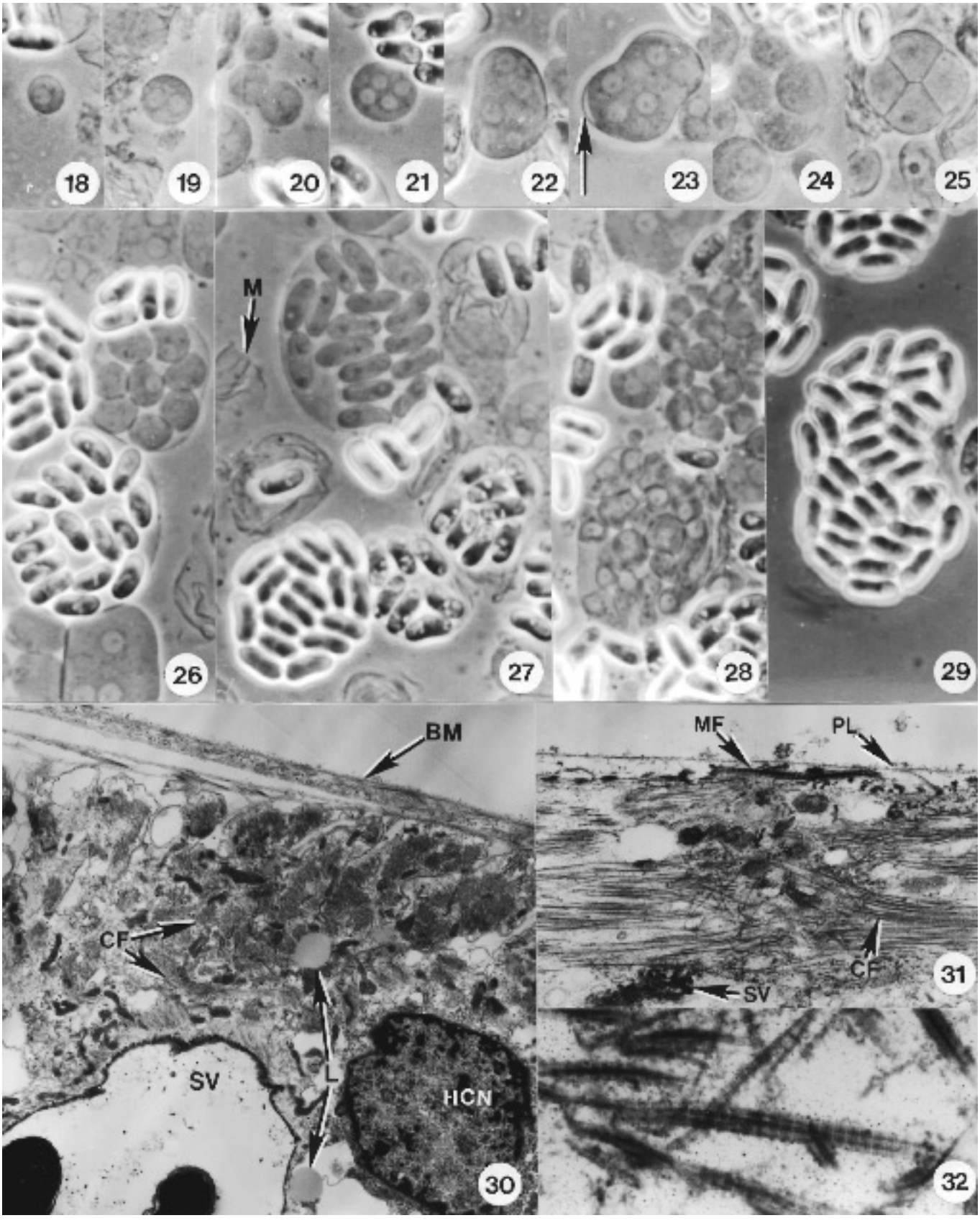
were particularly useful in determining the tissue affected. Thin sections were poststained with methanolic uranyl acetate followed by lead citrate. Thin sections were observed and photographed at an accelerating voltage of 75 kV with a Hitachi H-600 electron microscope.

An effort has been made in this contribution to use the terminology and classification recently proposed for the microsporidia by Sprague, Becnel, and Hazard (1992).

RESULTS

General Observations

Infected insects were moribund at the time of dissection. No obvious external signs of infection were evident. Field prevalence of infection was 6.25% ($n = 32$). Between 50 and 80% of the *L. m. capito* and *L. m. migratorioides* inoculated in several of the bioassay experiments developed infection. There was no evidence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of infection in the Melanoplinae grasshoppers fed spores.

Gross Pathology

Both field-collected and inoculated locusts showed the same gross appearances of infections. Advanced or heavy infections were easily detected upon dissection by the accumulation of large, white masses suspended in the hemocoel of the host which contained developmental stages and spores of the parasite. In a ventrally dissected locust, these accumulations appeared to be in association with the digestive tract or the body wall (Fig. 1). There was a considerable reduction in the amount of fat body in the infected locusts as determined by comparison of the diseased and healthy insects (Figs. 1 and 2). Evidence of an immune response (encapsulation, melanization) was never observed. Careful examinations revealed that the tissues surrounding the digestive tract were the main sites of development of the pathogen. Sections of the infected tissues demonstrated the symbiotic complex (xenoma) formed by the hypertrophic host cell(s) together with large numbers of developmental stages and spores of the parasite (Figs. 3 and 4). Histological sections of infected tissues indicated that these infected tissues were adipose tissue (fat body) (Figs. 3 and 4).

Light Microscopy Studies

Giemsa-stained preparations. All developmental stages observed were haplokaryotic (i.e., nuclei not diplokaryotic) and were spherical or mostly rounded in shape. The earliest developmental stages (schizonts) were small, uninucleate bodies, often in the process of karyokinesis (Fig. 5) that underwent binary division directly (Fig. 6). Schizonts apparently could develop into paucinucleate plasmodia (Figs. 7–9) with the potential to either break up into more schizonts or to develop (with an increase in the size and number of nuclei) into sporogonial plasmodia.

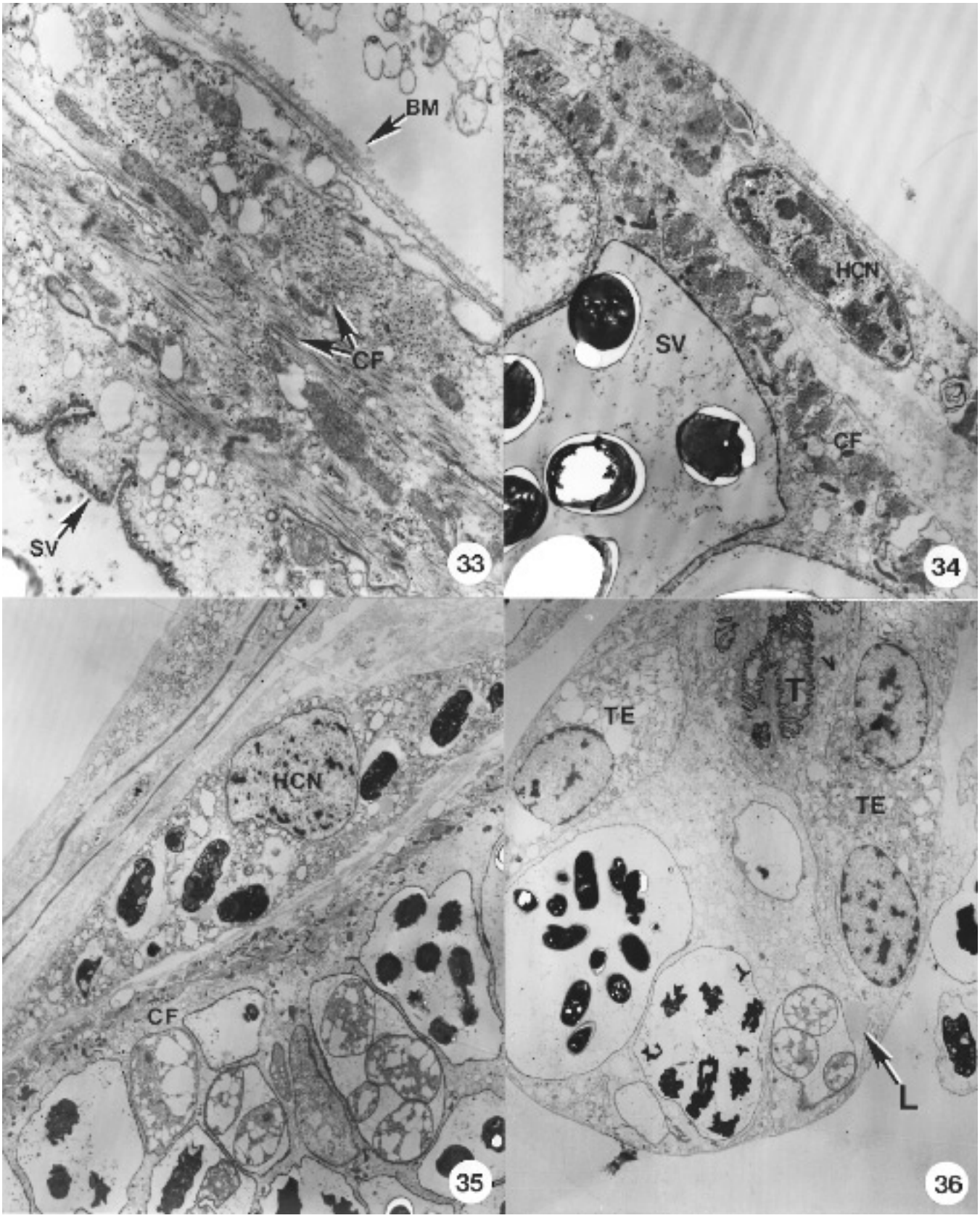
Plasmodia believed to be transitional to sporogony normally contained between 8 and 16 nuclei (Figs. 10–12). These transitional plasmodia grew in size and gradually became more vacuolated. The nuclei appeared to became segregated, primarily into groups of four apparently in preparation for plasmotomy (Fig. 12). Plasmodia with 32 nuclei could not be conclusively demonstrated.

Vesicles that contained eight rounded stages with dividing nuclei probably represented sporoblast mother cells (Fig. 14). The final products of sporogony were most frequently 16 sporoblasts or spores (Figs. 16 and 17) in sporophorous vesicles; eight spores in a sporophorous vesicle were less common (Fig. 15). The occurrence of sporophorous vesicles was inferred from spore groupings (Figs. 15 and 17) and only infrequently from the presence of an envelope (Fig. 14). Sporogonic stages with 32 nuclei were observed (Fig. 13) but it cannot be discounted that this may actually represent the division of two plasmodia with 16 nuclei each. Spores always appeared to be uninucleate and measured 6.9 ± 0.07 by 4.2 ± 0.05 μm (mean ± SE, fixed, $n = 50$).

Fresh mounts. As with the Giemsa-stained smears, the first stages observed were uninucleate and binucleate rounded bodies (Figs. 18 and 19), the latter frequently undergoing binary fission (Fig. 20). Paucinucleate plasmodia with simple plasmalemmas were common (Fig. 21). The first stages recognized as sporogonial were large multinucleated plasmodia with the beginnings of a thick outer membrane (Fig. 22). Soon after the membrane of the plasmodium was completely thickened, the plasmalemma began to retract simultaneously in different regions (Fig. 23). Interfacial envelopes and episporontal spaces became gradually more evident with progressive retraction of the plasmalemma. Sporogonial plasmodia commonly underwent plasmotomy to produce four bodies of equal size (Figs. 24 and 25). Since the most prevalent sequence during sporulation ended with sporophorous vesicles containing 16 spores (Figs. 26 and 27), it is presumed that each of the four products from plasmotomy normally contained four nuclei. Plasmotomy of an octonucleate plasmodium was much less frequent and probably resulted in two tetranucleate lobes that divided to produce sporophorous vesicles containing 8 spores (Fig. 27). Furthermore, the rare occurrence of sporophorous vesicles with 32 spores (Fig. 29) indicated that other types of plasmotomy were possible (i.e., plasmodia dividing into four octonucleate plasmodia?). In the dominant sequence, it appeared that each of the four plasmodia

FIGS. 18–29. Developmental stages and spores of *J. locustae* from fresh mounts. All, ×2000. (18) Uninucleate stage. (19) Binucleate stage. (20) Binary fission of binucleate stage. (21) Plasmodium with five nuclei visible. (22) Multinucleate plasmodium with thickened outer membrane. (23) Plasmodium in which the plasmalemma has started to retract (arrow) from the surface coat to form an interfacial envelope. (24,25) Plasmotomy of plasmodium resulting in four small plasmodia of equal size but with an undetermined (4?) number of nuclei. (26) Two vesicles, one containing eight sporoblast mother cells (presumably with two nuclei each), and the other, 16 spores. (27) Vesicles containing young and mature spores, two with 8 and two with 16. Note pieces of broken membranes (M) and the liberated spores. (28) Vesicle in which some stages (sporoblast mother cells?) appear to be dividing to form uninucleate sporoblasts. (29) Sporophorous vesicle with 32 spores.

FIGS. 30–32. Electron photomicrographs of the xenoparasitic complex as a result of infection by *J. locustae.* (30) Details of the xenoma wall limited by a basement membrane (BM) beneath which are collagen-like fibers (CF), lipid droplets (L), and a host cell nucleus (HCN). ×7500. (31) Details of the xenoma wall limited by a simple plasmalemma (PL) beneath which are collagen-like fibers (CF) and a portion of a muscle fiber (MF). SV, sporophorous vesicle. ×22,500. (32) Array of disrupted muscle fibers in the cytoplasm of the xenoma wall. ×75,000.



FIGS. 33–36. Electron photomicrographs of the xenoparasitic complex as a result of infection by *J. locustae.* (33) Details of a typical region of the xenoma wall. Basement membrane (BM); collagen-like fibers (CF); envelope of the sporophorous vesicle (SV). ×15,600. (34) Portion of the xenoma wall covered by an intact fat body cell. Host cell nucleus (HCN); region containing collagen-like fibers (CF); sporophorous vesicle (SV). ×6500. (35) Portion of the xenoma wall covered by an infected fat body cell (several lipid droplets evident in the cytoplasm). Host cell nucleus (HCN); region containing collagen-like fibers (CF); ×3000 (36). Portion of the xenoma with trachea and tracheal epithelium cells around which lies infected fat body. Trachea (T); tracheal epithelium (TE); lipid (L). ×1950.

resulting from plasmotomy divided to form two sporoblast mother cells (Fig. 26), probably each with two nuclei. Binary fission of these cells formed uninucleate sporoblasts (Fig. 28). Fresh mature spores (Figs. 27 and 29) were mostly elongate ovoid, sometimes slightly curved, and measured 8.3 ± 0.04 by 3.8 ± 0.04 μm (mean ± SE, $n = 50$). Sporophorous vesicles were remarkably persistent; even when ruptured, their remains were easily visible as folded membranes suspended among intact vesicles (Fig. 27).

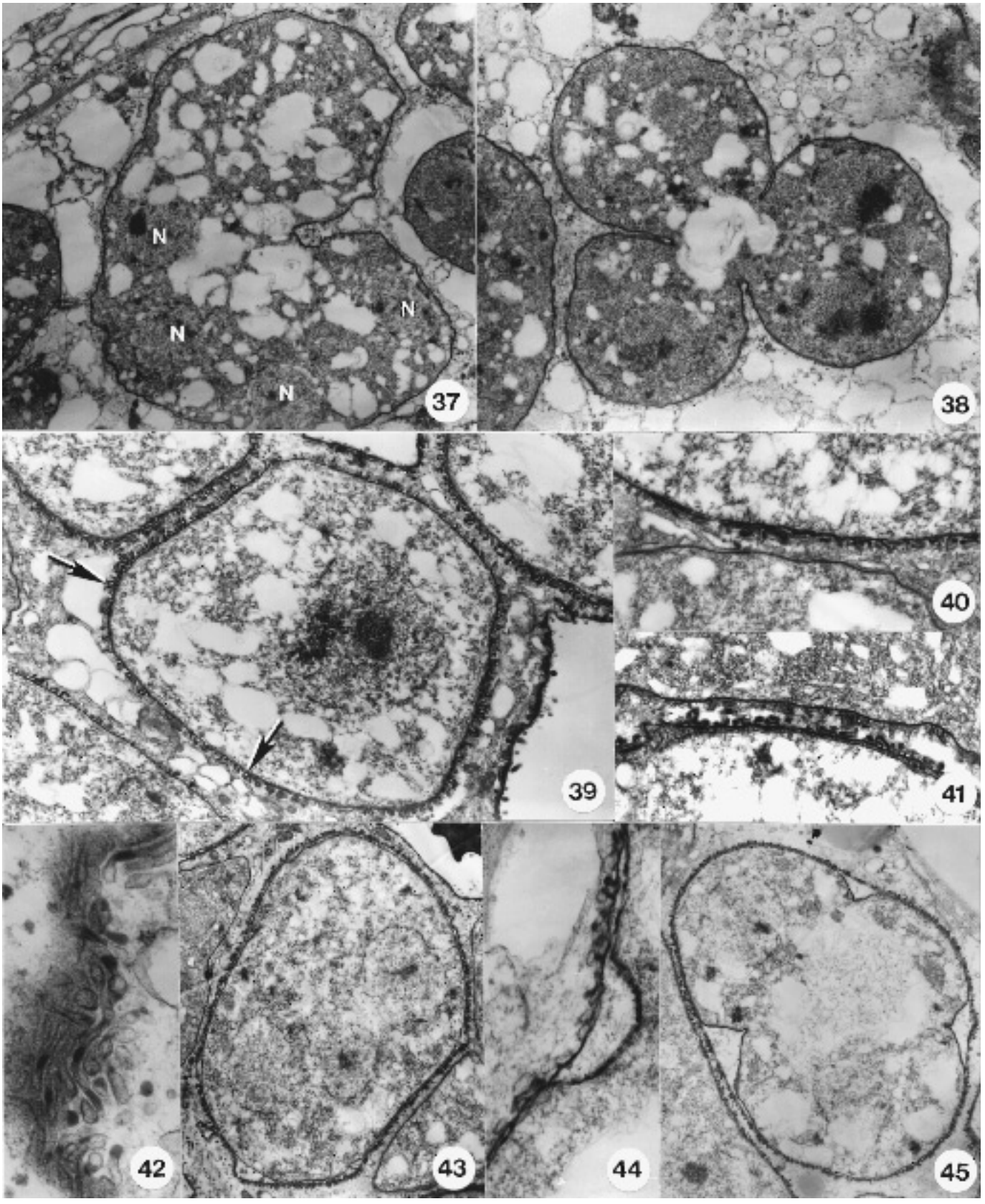
Electron Microscopy

Xenoparasitic complex. The formation of the xenoma is a complex process and the early stages of development were not observed. The initial observation that the xenomas were composed primarily of fat body cells was confirmed by the presence of lipid bodies and peripherally located nuclei (Fig. 30). These nuclei did not appear to be hypertrophied and it is presumed that the xenoma represented a syncytium formed by several cells. However, it is possible that the multiple nuclei represented the fragmentation of the nucleus from one cell.

The wall of the xenoma was typically composed of what appeared to be a basement membrane beneath which were numerous bundles of collagen-like fibers in concentric layers (Figs. 30 and 33). These layers ran perpendicular to one another as demonstrated by the presence of both transverse and longitudinal sections of the collagen-like fibers in the same field of view (Figs. 30, 33, and 34). The cytoplasm was rich in mitochondria and contained smooth walled channels and vesicles and flattened cisternae (Figs. 30, 33, and 34). Some regions of the xenoma wall (apparently degenerating) were limited by a single plasmalemma and composed of disorganized regions of collagen-like fibers and less frequently, muscle fibers (Figs. 31 and 32). Intact cells (suspected to be fat body cells based on the presence of lipid droplets) were sometimes found closely applied to the outside of the xenoma wall (Figs. 34 and 35). Occasionally, these cells were infected with parasite stages that appeared to develop into spores without the involvement of a sporophorous vesicle (Figs. 35 and 53). In localized regions, nerve cells, trachea, and tracheal epithelial cells were found to be associated with the xenoma. Several tracheal epithelial cells could often be seen in a single plane (Fig. 36), but these cells were rarely if ever infected. There was no obvious pattern of distribution of the different stages (i.e., stratification) of the parasite within the xenoma.

The parasite. The nuclear condition was always haplokaryotic. Some plasmodia, perhaps transitional, contained many nuclei and had a single, relatively thick, electron-dense outer membrane (Figs. 37 and 38). The cytoplasm was highly vacuolated and contained an abundance of ribosomes (Figs. 37 and 38). A first division of these plasmodia was by plasmotomy (Fig. 38). Similar plasmodia developed numerous dense appendages in physical continuity with the outer surface of the plasmalemma (Fig. 39), thereby becoming recognizable as sporogonial. These appendages were tubular in structure with terminal or intermediate blebs and eventually covered the entire surface of the plasmodium (Figs. 39–42). At that point, a doubling of the plasmalemma occurred (Figs. 40 and 41). The actual mechanism for this doubling was not established, but it progressed until the whole plasmodium was surrounded by two membranes; the outer one with appendages and the inner one simple (Figs. 40, 41, and 43). The sporogonic division was plasmotomy, starting with localized retraction of the inner membrane (Figs. 43–45) which always occurred immediately after the doubling of the original plasmalemma. Retraction of the inner membrane proceeded simultaneously in several different areas of the plasmodium (Figs. 43–46). At this time, episporontal spaces were electron-lucent and devoid of any obvious structural features. Numerous appendages on the outer membrane (the interfacial envelope) persisted and resulted in a considerable increase in the surface contact between the parasite and host cells (Figs. 43–49). The outcome of the plasmotomy was four tetranucleate plasmodia, each of which divided into two sporoblast-mother cells (Fig. 47). The latter underwent a final division (binary fission) to form two uninucleate sporoblasts (Fig. 48). The appendages on the outer surface of interfacial envelopes persisted throughout the sporulation process. Episporontal spaces were mostly devoid of any particular morphological features. However, in some vesicles an array of tubules was uniformly distributed in the episporontal space (Fig. 49). The tubules were relatively long, appeared to be interconnected (Fig. 50) and were not continuous with the wall of the sporoblasts. Infrequently, assemblages of unique structures were found occupying small areas of the episporontal space (Fig. 51). These structures were highly electron-dense and appeared mostly like incomplete rings embedded in a dense matrix.

Spores were uninucleate and possessed a well-developed polaroplast that occupied the anterior half of the spore (Fig. 52). The polaroplast consisted of lamellar and cystic or vesicular portions. A vesiculated area at the other end represented the posterior vacuole largely filled with posterosomes and other vesicular matter. Rough endoplasmic reticulum was common in the vicinity of the nucleus. The polar filament was isofilar and singly coiled with 12 turns. The endospore was only moderately thick while the exospore was very thin and unornamented. Occasionally, some free spores (i.e., not within sporophorous vesicles) were seen in the cytoplasm of the outer regions of the xenoma (Fig. 53). These spores appeared to have the same ultrastructural features as the ones within enve-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lopes, including the number of turns in the polar filament coil.

DISCUSSION

This is the first report of a microsporidium without diplokaryotic stages from an orthopteran host. Information presented here has demonstrated that this new microsporidium is haplokaryotic throughout development, produces polysporophorous vesicles of parasite origin and there is conspicuous xenoma formation.

The terms "conspicuous" or "distinctive" xenoma formation were utilized by Sprague *et al.* (1992) to distinguish those host–parasite associations "that exhibit spectacular morphological modifications." The xenoma formation of the species described in this study appears to be similar to the syncytial type as described by Weiser (1976) which was also bound by a basement membrane wall. However, this new species exhibits gross morphological modifications similar to the "*Glugea*-cysts" as described by Weiser (1976) and demonstrated for the type species *G. anomala* (see Canning *et al.,* 1982). Conspicuous xenoma formation of this type is widespread among microsporidia infecting fish (Lom and Dyková, 1992), but is less common for microsporidia infecting arthropods. A microsporidium with conspicuous xenoma formation was reported by Gasc *et al.* (1976) and Loubes *et al.* (1976) from the muscle tissue of millipedes (Myriapoda: Polydesmidae) and described as *G. habrodesmi* Loubes, Maurand, Gasc, and Bouix (1976). Judging from the published electron micrographs, *G. habrodesmi,* like the microsporidium in *L. m. capito,* fits well in the family Glugeidae. Its generic status, however, is arguable because presporogonic development with cylindrical plasmodia and involvement of membranes of host endoplasmic reticulum were not observed, and the host is an arthropod. Moreover, the sporogonic development of *G. habrodesmi* is consistent with the new microsporidium in *L. m. capito* and further studies could lead one to recognize that they are congeneric.

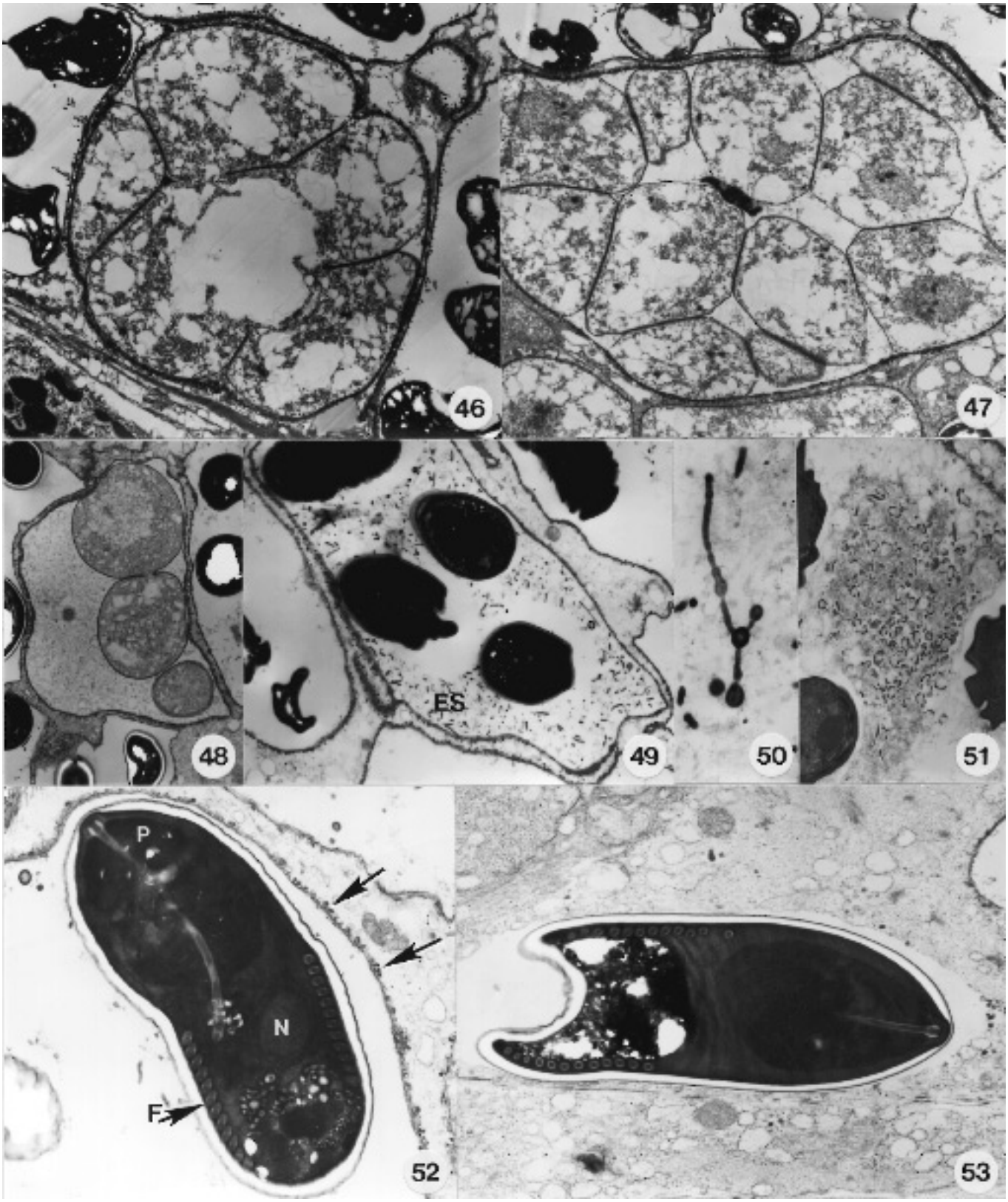
Based on the information presented here, this new microsporidium is most closely aligned to members of the family Glugeidae (Sprague *et al.,* 1992). Three genera, *Glugea, Loma,* and *Glugoides* are currently included within Glugeidae. The microsporidium from *L. m. capito* differs from the type species *G. anomala* in lacking cylindrical plasmodia during presporogony and without involvement of the cisternae of host cell endoplasmic reticulum in the formation of the interfacial envelope (Canning *et al.,* 1982). Since Morrison and Sprague (1981) also described layers of host cell endoplasmic reticulum around presporogonic stages, and demonstrated the occurrence of moniliform plasmodia in *Loma morhua* (type species), the organism studied here can also be excluded from the genus *Loma.*

In addition to the developmental differences discussed above, it is well established that species of *Glugea* and *Loma* are primarily restricted to fish (Canning and Lom, 1986; Lom and Dyková, 1992). Moreover, the site of infection could also be characteristic with species of *Glugea* in mesenchyme cells and species of *Loma* in gills (Sprague *et al.,* 1992). Although it could be argued that host affinity and specificity is of limited taxonomic value for the microsporidia, the large taxonomic gap between fish and locusts suggests that these characters provide additional support for the distinction of this new microsporidium from species of *Glugea* and *Loma.*

Recently, the newly created genus *Glugoides,* represented by the gut parasite *G. intestinalis* (Chatton, 1907) from a Cladocern host, was placed into the family Glugeidae (see Larsson *et al.,* 1996). This genus is similar to the new species described here by having all life cycle stages with isolated nuclei, the production of polysporphorous vesicles, and a large and variable number of sporoblasts per sporont. It differs in that all stages are within a parasitophorous vesicle with sporogonial stages further isolated by a sporophorous vesicle but there is no xenoma formation. In addition, reproduction in *G. intestinalis* during merogony and sporogony is by plasmotomy. In the new species described here, presporulation reproduction is by binary and multiple fission and sporogony results in sporoblast mother cells that divide to produce sporoblasts. In addition to these developmental differences, *G. intestinalis* and the new species described here invade different tissues, are found in widely separate host groups, and elicit distinctly different host responses to infection.

Based on the characteristics for the microsporidium isolated from *L. m. capito* and its distinctiness from other genera, a new genus, *Johenrea,* with *Johenrea locustae* as the type species, is proposed. The generic

FIGS. 37–45. Electron photomicrographs of developmental stages of *J. locustae.* (37) A highly vacuolated transitional (sporogonial) plasmodium with at least four nuclei (N) and a thickened electron-dense outer membrane. ×6600. (38) Plasmotomy of a transitional (sporogonial) plasmodium with possibly 16 nuclei (one lobe not visible). ×9600. (39) Sporogonial plasmodium with a single membrane ornamented with dense, tubular appendages (arrows). ×13,500. (40) Surface membranes of two sporogonial plasmodia; the one on the bottom is thickened but still single, the one on the top with tubular appendages. ×20,000. (41) Parts of single (top) and doubled (bottom) membranes of two plasmodia separated by host cell cytoplasm. Note that appendages are already formed on the former and are still present in the latter. ×20,000. (42) Detail of the tubular appendages on the surface of the outer membrane (i.e., interfacial envelope) of a sporogonial plasmodium. ×35,000. (43) A sporogonial plasmodium subsequent to complete doubling of the plasmalemma. ×6000. (44) Retraction of the inner plasmalemma from the interfacial envelope at the beginning of plasmotomy. ×30,000. (45) Further retraction of the inner membrane of a plasmodium starting to undergo plasmotomy (the first sporogonic division) to produce four small plasmodia. ×6000.



FIGS. 46–53. Electron photomicrographs of late sporogony and spores of *J. locustae.* (46) Second division of a sporogonial plasmodium in progress to form eight sporoblast mother cells before the first is complete. ×9000. (47). Sporont mother cells after completion of the second sporogonic division of a sporogonial plasmodium. ×4800. (48). Vesicle showing what seems to be the final (third) division of a sporoblast mother cell dividing into two sporoblasts. ×5000. (49). Vesicle containing immature spores and an abundance of tubular structures in the episporontal space (ES). ×5000. (50). Tubular structure in the episporontal space. ×17,000. (51). Rare, dense structures with the shape of incomplete rings in the episporontal space of a vesicle. ×10,000. (52). Mature uninucleate spore inside a sporophorous vesicle. Note the nucleus (N), the polaroplast (P), and the coils of the polar filament (F). Also see the appendages (arrows) still present on the surface of the sporophorous vesicle. ×15,000. (53). Mature uninucleate spore not within a sporophorous vesicle. ×10,000.

name is in honor of Dr. John E. Henry, a pioneer in the study of orthopteran diseases. Diagnosis is as follows.

Johenrea n.g.

All life cycle stages haplokaryotic. Multiplication by way of both a schizogonic phase as well as by a sporulation phase producing polysporophorous vesicles of parasite origin. Schizonts divided either directly or with the intervention of paucinucleate plasmodia into additional schizonts. Sporogonial plasmodia divided by plasmotomy to form sporoblast mother cells that divided by binary fission. Sporogonial plasmodia elaborated an interfacial envelope within which they underwent sporogony. Sporophorous vesicles normally contained 16 spores but also 8 and rarely 32 spores. Spores with typical microsporidian structure. Conspicuous xenoma formation.

Johenrea locustae n.sp.

Type host. *L. m. capito* (Saussure, 1884) (Orthoptera: Acrididae: Oedipodinae). Other known host (experimental): *L. m. migratorioides* (Reiche and Fairmaire, 1850).

Transmission. Per os.

Site of infection. Fat body.

Interface. During presporulation, parasite in hyaloplasm of the cell. During transition to sporogony, the plasmalemma of the parasite doubles, the outer membrane becoming a persistent interfacial envelope.

Other parasite–host cell relations. Conspicuous xenoma formed. The xenoma was composed mainly of fat body cells limited by a basement membrane. The outer region of the xenoma (xenoma wall) was primarily composed of bundles of collagen-like fibers in concentric layers that ran perpendicular to one another. This region was of variable thickness depending upon the location and the maturity of the xenoma. In localized regions, tracheal epithelial cells, nerve cells, and muscle fibers were associated with the xenoma wall. There was no obvious pattern of distribution of the different stages (i.e., stratification) of the parasite within the xenoma.

Schizogony. Proliferation is generally by binary fission of uninucleate schizonts, probably sometimes multiple fission of paucinucleate plasmodia (4 or 8 nuclei?) resulting from delayed cytokinesis.

Schizogony–sporogony transition. Mixed with the schizogonic stages in the hyaloplasm, and presumably arising from them, are plasmodia with 8, 16 (most common), and rarely 32 nuclei, some (if not most) of them being stages in development of sporogonial plasmodia. Plasmodia distinguishable as sporogonial when they start to elaborate an interfacial envelope. The outer surface of the plasmalemma developed numerous dense appendages and eventually covered the entire surface of the plasmodium. A doubling of the plasmalemma then occurred and progressed until the whole plasmodium was surrounded by two membranes; the outer one with appendages (the interfacial envelope) and the inner one simple (the plasmalemma).

Sporogony. Typically, a sporogonial plasmodium with 16 nuclei undergoes a rather regular progression of divisions in three steps. First, a plasmotomy (beginning before, during, or after elaboration of an interfacial envelope and involving a four-lobe stage in the plasmodium) ends with four small tetranucleate plasmodia. Next, and often starting before the first division is finished, each tetranucleate plasmodium divides into two binucleate sporoblast mother cells. Finally, each of the latter divides into two sporoblasts. Thus, 16 sporoblasts are produced within a vesicle. Sometimes a sporogonial plasmodium containing 8 or 32 nuclei produces 8 or 32 sporoblasts.

Spore. Uninucleate. Elongate ovoid (sometimes slightly curved) 8.3 ± 0.04 by 3.8 ± 0.04 μm (fresh, mean ± SE, $n = 50$) and 6.9 ± 0.07 by 4.2 ± 0.05 μm (fixed, mean ± SE, $n = 50$). Polar filament isofilar, singly coiled with 12 turns. Well-developed polaroplast and posterior vacuole.

Type locality. Surroundings of Ambalavenoka, about 50 km north of Tulear (Toliara) in southwestern Madagascar.

Deposition of type specimens. Giemsa-stained slides were deposited under USNM numbers 47829 and 47830 in the International Protozoan Type Slide Collection.

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REFERENCES

Canning, E. U., and Lom, J. 1986. "The Microsporidia of Vertebrates." Academic Press, London.

Canning, E. U., Lom, J., and Nicholas, J. P. 1982. Genus *Glugea* Thélohan 1891 (Phylum Microspora): Redescription of the type species *Glugea anomala* (Moniez 1887) and recognition of its sporogonic development within sporophorous vesicles (pansporoblast membranes). *Protistologica* **18,** 193–210.

C.O.P.R. (Centre for Overseas Pest Research, Ed.). 1982. "The Locust and Grasshopper Manual." London.

Gasc, C., Loubes, C., Maurand, J., and Bouix, G. 1976. Sur une nouvelle espece de Microsporidie, parasite de Myriapodes Diplopodes du Sud-Dahomey. *Acta Trop.* **33,** 169–176.

Henry, J. E. 1985a. Effect of grasshopper species, cage density, light intensity, and method of inoculation on mass production of *Nosema locustae* (Microsporida: Nosematidae). *J. Econ. Entomol.* **78,** 1245–1250.

Henry, J. E. 1985b. *Melanoplus* spp. *In* "Handbook of Insect Rearing," (P. Singh and R. F. Moore, Eds.), Vol. 1, pp. 451–464. Elsevier, Amsterdam.

Henry, J. E., Tiahrt, K., and Oma, E. A. 1973. Importance of timing, spore concentrations, and levels of spore carrier in applications of *Nosema locustae* (Microspora: Nosematidae) for control of grasshoppers. *J. Invertebr. Pathol.* **21,** 263–272.

Larsson, J. I. R., Ebert, D., Vávra, J., and Voronin, V. N. (in press). Redescription of *Pleistophora intestinalis* Chatton, 1907, a microsporidian parasite of *Daphnia magna* and *Daphnia pulex* with establishment of the new genus *Glugoides* (Microspora, Glugeidae). *Eur. J. Protistol.* **32,** 251–261.

Lom, J., and Dyková, I. 1992. "Protozoan Parasites of Fishes." Elsevier, Amsterdam.

Loubes, C., Maurand, J., Gasc, C., and Bouix, G. 1976. Etude ultrastructurale de *Glugea habrodesmi* n. sp., (Microsporida, Glugeidae) parasite des Myriapodes *Habrodesmus fals* Cook et *Oxydesmus granulosus* Palisat de Beauvois (Myriapoda, Polydesmidae). *Protistologica* **12,** 435–450.

Morrison, C. M., and Sprague, V. 1981. Electron microscopical study of a new genus and species of microsporida in the gills of Atlantic cod *Gadus morhua* L. *J. Fish Dis.* **4,** 15–32.

Poinar, G. O., and Thomas, G. M. 1984. "Laboratory Guide to Insect Pathogens and Parasites." Plenum, New York.

Sprague, V., Becnel, J. J., and Hazard, E. I. 1992. Taxonomy of Phylum Microspora. *Crit. Rev. Microbiol.* **18**(5/6), 285–395.

Steedman, A. (Ed.). 1990. "Locust Handbook," (2nd ed.) Overseas Development Natural Resources Institute (ODNRI), London.

Wang, L. Y., Streett, D. A., and Henry, J. E. 1991. *Nosema montanae* n. sp. (Microsporida: Nosematidae), a parasite from the grasshopper *Melanoplus packardii* (Orthoptera: Acrididae). *J. Invertebr. Pathol.* **58,** 211–218.

Weiser, J. 1976. The *Pleistophora debaisieuxi* xenoma. *Z. Parasitenk.* **48,** 263–270.