refractile colorless wall. These oocysts are considered on the basis of size to be those of *E. tachyglossi*. Occasionally, smaller oocysts, about 16 in diameter were seen in epithelial cells on villi, but it is not known whether these were *E. echidnae* or merely chords taken through a larger *E. tachyglossi* oocyst. In heavily infected mucosa, the crypts of Lieberkühn were elongate in comparison with adjacent, less heavily infected tissue. Infected epithelium did not seem to have been as susceptible to autolytic slough as the uninfected. No significant inflammation was noted. Similar findings were made in the Tasmanian animals although one had a diffuse subacute inflammatory reaction in the lamina propria of the small intestine.

DISCUSSION

Octosporella hystrix is the first species of the genus to be recognized in mammals. Hitherto, it has been reported only from reptiles in India (2) and Turkmen SSR (1). Oocysts were present in large numbers in the feces of only one animal from mainland Australia. Spurious parasitism may have occurred, but was considered unlikely since echidnas eat only ants and termites, which would have had to have been fortuitously extremely heavily infected to have caused so many oocysts to be in the feces of the echidna. Endogenous development was not investigated.

Both *E. tachyglossi* and *E. echidnae* were prevalent in our small sample of Tasmanian echidnas. *Eimeria tachyglossi* was invariably more numerous in individual specimens. The species are separated on the basis of oocyst size, thickness of oocyst wall, and shape and size of sporocysts.

The oocysts in the feces of the dead Victorian echidna fall within the size ranges of the two species of *Eimeria* described, and the observations of endogenous stages indicate that at least *E. tachyglossi* may parasitize the lower two-thirds of the small intestine. Whether *E. echidnae* was also present here is difficult to resolve, but coccidia were not seen in other parts of the gut or its accessory organs.

The pathogenicity of coccidia in the echidna is unknown. In two of the three dead echidnas there was concurrent illness that may have accounted for the death of the animals. This included in one case infestation with thousands of immature *Aponomma concolor* and focal hepatic necrosis of unknown etiology. Further information is required to determine whether these coccidia are pathogenic in the echidna.

LITERATURE CITED

1. Ovezmukhammedov, A. 1975. [*Octosporella sanguinolenti* sp. n. A new species of coccidia from *Agama sanguinolenta* Pallas.] *Izvest. Akad. Nauk. Turkmen SSR, S. Biol. Nauk.*, **4**: 87–88. (in Russian)

2. Ray, H. N. & Raghavachari, K. 1942. Observations on a new coccidium, *Octosporella mabuiae* n. gen., n. sp., from the intestine of *Mabuia* sp. *Proc. 28th Ind. Sci. Congr.* Abstr. 170. (Cited in: Pellerdy, L. P. 1974. *Coccidia and Coccidiosis.* 2nd ed. Verlag Paul Parey, Berlin and Hamburg, p. 119.)

Received 26 V 83; accepted 28 II 85

J. Protozool., 32(3), 1985, pp. 525–535

Two New Species of *Nosema* (Microsporida: Nosematidae) from the Mexican Bean Beetle *Epilachna varivestis* (Coleoptera: Coccinellidae)[1,2]

W. M. BROOKS,* E. I. HAZARD,† and J. BECNEL****

**Department of Entomology, North Carolina State University, Raleigh, North Carolina 27695 and*
***Gulf Coast Mosquito Research, Agricultural Research Service, U.S. Department of Agriculture, Lake Charles, Louisiana 70601*

ABSTRACT. Two new species of the genus *Nosema* (Microsporida: Nosematidae) are described from the Mexican bean beetle, *Epilachna varivestis* (Coleoptera: Coccinellidae) and their life cycle stages studied by light and electron microscopy. Both species are monomorphic and disporous; they develop in direct contact with the cytoplasm of host cells and the nuclei of all stages are diplokaryotic. The more virulent species produces systemic infections most extensively in the adipose tissue, muscles, and Malpighian tubules of larvae and also invades the reproductive tissues of adult beetles. During merogonic development, it forms chains of diplokaryotic meronts. The fine structure of the sporoblast nuclei shows clumped material in the pole of each nucleus opposite their common plane of apposition. Spores are straight to slightly curved and ovocylindrical in shape and they measure 5.3 ± 0.13 × 2.1 ± 0.03 μm. The less virulent species also invades most host tissues but does not develop in the midgut epithelium; the Malpighian tubules are the principal site of its development and it also invades the ovaries and testes of adult beetles. Merogony occurs exclusively as the result of binary fission of diplokaryotic meronts. The plasmalemma of the meronts is covered with a thin deposit of exospore material upon which are located closely packed tubules that encircle the body transversely. A thickened deposit of exospore material on the surface of the diplokaryotic sporonts later obscures these tubules. Other tubules occur free in the host cell cytoplasm or attached to the plasmalemma of meronts and sporonts. Secretory granules also occur free or in chains in the host cytoplasm and are probably produced from the surface of the sporoblasts. Sporoblasts also contain an unusual cup-shaped organelle associated with a dense body, which is apparently involved in the formation of the polar tube and its associated organelles in the anterior part of the spore. Spores are ellipsoidal to slightly pyriform and measure 4.7 ± 0.06 × 2.6 ± 0.03 μm.

THE Mexican beetle (MBB), *Epilachna varivestis* Mulsant, is one of few species of the predominantly predacious ladybird family Coccinellidae that is phytophagous and is considered to be a serious insect pest of bush beans and soybeans in the US (32, 38). As few pathogens are known from the MBB (2, 9), efforts to control this pest biologically have focused primarily on the importation and colonization of various tachinid and hymenopterous parasites (24, 32). Cantwell & Cantelo (5) determined that the MBB was susceptible to a preparation of *Bacillus thuringiensis* containing a heat-stable exotoxin but such formulations of *B. t.* are not yet registered for use in the US.

Other potential microbial control agents include a few species of bacteria and fungi of uncertain taxonomic status that were reported associated with the MBB in the early literature (10,

[1] Our thanks are extended to Mr. Jimmy Cranford and Mr. Tokuo Fukuda for their technical assistance during this investigation. We also appreciate the assistance of Ms. Brenda Morrison, who prepared the plates for the manuscript.

[2] Paper number 9481 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, North Carolina 27695.

† Sadly deceased on April 8, 1985.

11, 19, 20). More recently, Adams et al. (1) found two types of virus-like particles that closely resemble rhabdoviruses in MBB larvae, and Quattlebaum & Carner (30) reported the presence of a possible new fungus in the genus *Beauveria*. Brooks et al. (4) recently published a note on the occurrence of three species of microsporidia, two in the MBB and one in the bean leaf beetle, *Ceratoma trifurcata* (Forster), which occur as pests of soybeans. The present paper describes, based on light and electron microscopy, the two species of microsporidia associated with the MBB. They were referred to previously as MBB sp. No. 1 and MBB sp. No. 2 (4). Both species have been found in field-collected and laboratory colonies of the beetles.

MATERIALS AND METHODS

Infected MBB larvae and adults were obtained by allowing newly hatched larvae to feed for 48 h on lima bean leaf discs surface-contaminated with an aqueous suspension of semi-purified spores of each microsporidium. These larvae were maintained subsequently at 26.6°C in petri dishes (1–5 larvae per dish) with fresh bean leaves, which were changed daily. At various intervals post-treatment, larvae or adults were smeared individually on glass slides and stained with 10% (v/v) solution of Giemsa (pH 7.4). Various life-cycle stages were also examined in preparations stained with Lacto-Aceto-Orcein (LAO) according to the technique of Hazard & Brookbank (15).

For electron microscopy, infected individuals were dissected in 2.5% (v/v) glutaraldehyde buffered in 0.1 M sodium cacodylate (pH = 7.4) with calcium chloride added. After 30 min, tissues were cut into smaller pieces and fixation was continued for about 2.5 h at room temperature. Tissues were post-fixed for 2 h in 1% (w/v) osmium tetroxide, dehydrated through a graded ethanol series into acetone, and embedded in Eponn-Araldite (23). Sections were stained in methanolic uranyl acetate followed by lead citrate (31) and examined with a Hitachi HS-8 electron microscope at 50 kV.

For histological observations, infected individuals were fixed for 24 h in acetic formalin, embedded in Paraplast, and sectioned longitudinally at 8 μm. Sections were stained with Heidenhain's hematoxylin and eosin.

Host range studies were conducted by exposing neonate larvae and/or adults of several species of Lepidoptera and Coleoptera to both species of microsporidia. The insects were exposed singly or in groups of five to leaf discs of appropriate plant foliage treated with known doses of spores in aqueous suspension. After 48 h, the insects were maintained on fresh foliage or on an artificial diet at 26.7°C for a 2 to 3 week period prior to examination. Relative susceptibility was determined by comparing the percent infection to that obtained with a similarly treated group of MBB larvae.

RESULTS

Nosema epilachnae n. sp.

Light microscopy. Meronts stained with Giemsa were typically spheroid to ovoid with diplokaryotic nuclei (Figs. 1, 2). The predominant merogonic forms present were binucleate meronts that divided repeatedly by binary fission to produce two daughter cells. Spherical meronts containing four diplokarya were also common (Fig. 2). These appeared to divide by budding (Figs. 3, 4) or, through a gradual process of cytokinesis, formed elongated buds (Fig. 5) that gave rise to four diplokaryotic cells. The paired nuclei in meronts undergoing elongation were arranged transverse to the longitudinal axis of the meront (Fig. 5) while the nuclei in meronts undergoing budding were arrayed end to end (Figs. 3, 4). Chains of merogonic cells containing four to eight diplokarya (Figs. 6–8) were also formed by synchronous nuclear divisions. These eventually produced four to eight daughter cells. The nuclei in these meronts were typically end to end with respect to the long axis of the chain. The meronts stained homogeneously except for a clear space around each of the deeply stained diplokarya. Spherical meronts with nuclei in diplokaryotic arrangement were also frequently noted in LAO preparations but no evidence of karyogamy or meiosis was seen.

The cytoplasm of stages presumed to be sporonts was lightly stained with Giemsa and exhibited various degrees of vacuolization (Figs. 9–12). Sporonts were fusiform with paired nuclei whose appearance ranged from being essentially like meronts to very irregular in outline and unevenly stained. They occurred singly or in various stages of binary fission (Figs. 11, 12). Sporonts were most easily distinguished from meronts on slides where infrequently the vegetative forms occurred together as a mass near the debris of single, infected host cells (Fig. 12). Although stages clearly identifiable as sporonts were not seen undergoing chain division, vegetative stages apparently in transition from meronts to sporonts occurred singly or occasionally as chains of four diplokaryotic cells (Fig. 13). These stages were ovocylindrical to slightly fusiform and stained less intensely than typical meronts in chains. It is assumed that following their separation by cytokinesis these stages divide at least once to produce two sporoblasts.

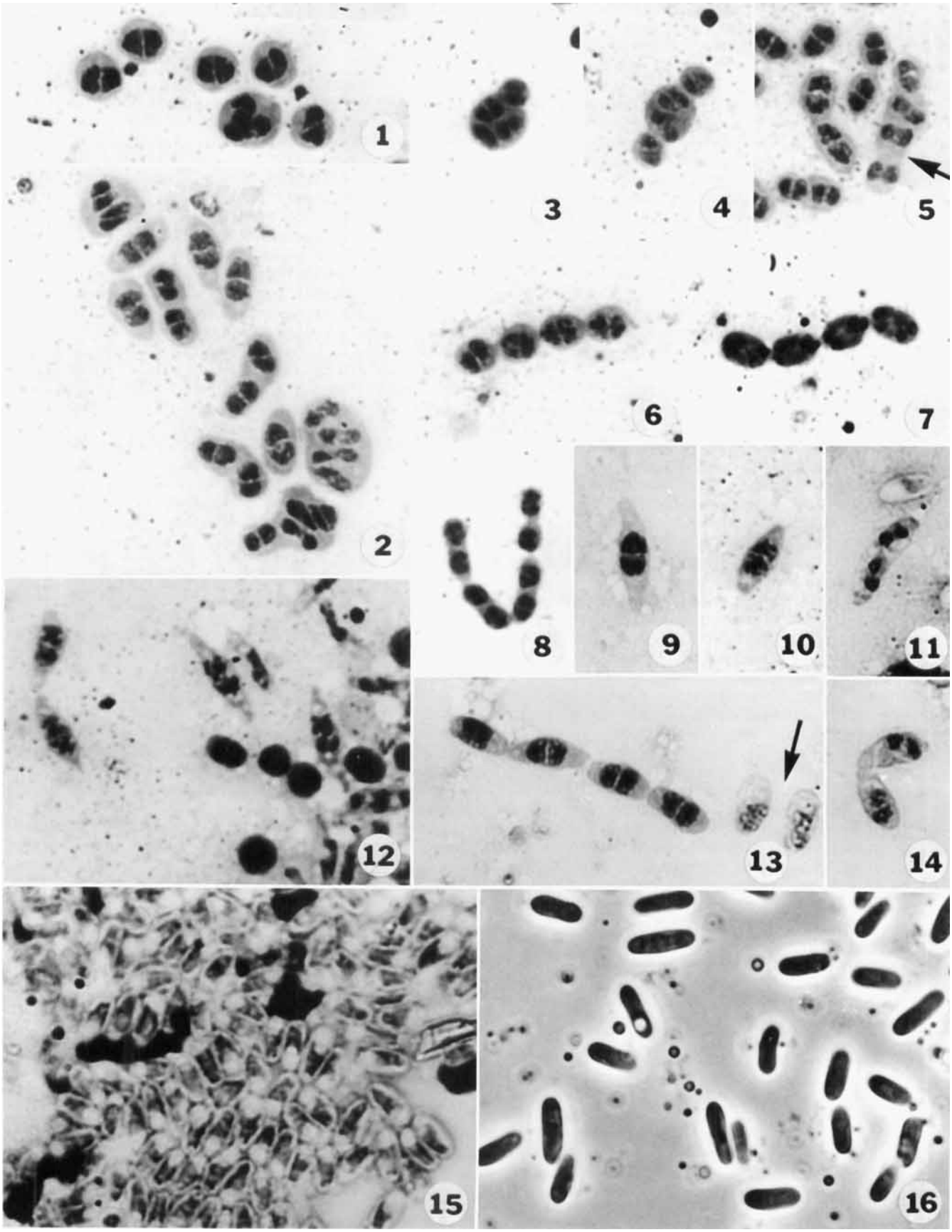
Sporoblasts (Figs. 13, 14) were ellipsoidal in shape and were also characterized by their lightly stained and vacuolated cytoplasm. Sporonts and sporoblasts were not easily distinguished from meronts in LAO preparations but their diplokaryotic nature could be seen in late-stage sporoblasts.

Although the spores were not obviously binucleate in Giemsa (Fig. 15) or LAO (Fig. 16) preparations, their diplokaryotic nature was confirmed in electron micrographs. Spores were ovocylindrical in shape and in wet-mount preparations measured 5.3 ± 0.13 × 2.1 ± 0.03 μm as reported previously (4). Polar tubes (extruded by mixing spores in aqueous suspension with H_2O_2) had a mean length of 84 μm.

Electron microscopy. Meronts were similar in shape to those observed by light microscopy and appeared as simple cells with diplokaryotic nuclei, flattened cisternae of rough endoplasmic reticulum (RER), and free ribosomes surrounded by a simple plasmalemma that was in direct contact with the cytoplasm of the host cell (Fig. 17). The nuclei were always diplokaryotic and the nucleoplasm was uniformly granular or contained a distinct area (nucleolus) of clumped chromatin. Spindle plaques closely associated with the nuclear membrane and spindle microtubules

→

Figs. **1–16**. Developmental stages of *Nosema epilachnae* as seen in Giemsa-stained smears (Figs. 1–15) and LAO-stained smears (Fig. 16). ×2000. **1**. Early diplokaryotic meronts. **2**. Diplokaryotic meronts with 2, 4, and 8 nuclei; several undergoing binary fission. **3**, **4**. Budding octonucleate meronts. **5**. Dividing meronts and octonucleate meront (arrow) undergoing elongation. **6**. Chain of four, diplokaryotic meronts. **7**. Merogonic chain in which the nuclei of each individual meront are undergoing karyokinesis. **8**. Chain of eight, diplokaryotic meronts. **9**, **10**. Diplokaryotic sporonts. **11**. Sporont undergoing binary fission. **12**. Group of spherical, darkly stained meronts and fusiform sporonts. **13**. Sporoblasts (arrow) and merogonic chain forming sporonts. **14**. Sporont producing sporoblasts. **15**. Mature spores. **16**. Fresh spores.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

were occasionally observed in dividing meronts. Despite their common appearance in Giemsa-stained smears, long chains of diplokaryotic meronts were seldom observed in the two-dimensional sections of material examined with the electron microscope.

Sporonts were characterized by an electron-dense coat on their surface (Fig. 17) which developed into the exospore layer of the spore wall. Vegetative stages in apparent transition from meronts to sporonts (Fig. 18) have an electron-dense coat formed only at one end. The sporonts were otherwise structurally similar to meronts except that free ribosomes were generally much less abundant while bound ribosomes were more prominent on the outer surface of the ER membranes. No evidence of a Golgi apparatus was observed in either sporonts or meronts. Dividing sporonts were common (Figs. 17, 19), and their nuclei contained spindle plaques and microtubules radiating from the plaques towards electron-dense structures (apparently chromosomes) in the nucleoplasm. Each sporont appeared to divide at least once (Fig. 19), giving rise to stages that ultimately developed into sporoblasts.

Sporoblasts were usually elongate and were characterized by a crenated outline and the presence of primordia of future spore organelles (Fig. 20). An unusual but also characteristic feature of the sporoblast was an electron-dense mass of material which occurred in the pole of each nucleus opposite their common plane of apposition (Fig. 20). Similar material was not observed in the nuclei of any other developmental stage. The sporoblast cytoplasm also contained free ribosomes, RER, a posterior vacuole, and early stages in the formation of the polar tube closely associated with a network of electron-dense material, representing the Golgi apparatus.

The interior of most mature spores was usually too electron-dense to visualize the various spore organelles. In some spores, however, it was possible to confirm the presence of the diplokaryon, 10–12 turns of the polar tube, a posterior vacuole, organized ribosomes, and a typical trilaminar spore wall (Fig. 21). The electron-transparent inner layer (the endospore) gradually thickened with spore maturity. The exospore was an electron-dense, relatively thin layer somewhat crenated in outline while the innermost layer, the plasmalemma, appeared as a very thin membrane limiting the spore contents.

Histological observations. The high virulence of this microsporidium for the MBB was readily apparent from the extensive and systemic infections produced in both larval and adult beetles. The infection was particularly heavy in the adipose tissue, muscles, and Malpighian tubules of larvae. In advanced larval infections, spores were also seen in nerve ganglia and in tracheal, epidermal, and midgut cells. In some sections of the adipose tissue, microsporidian development was accompanied by a mild inflammatory response marked by hemocytic infiltration and nodule formation. In addition to the extensive development of the microsporidium in these same tissues in adult beetles, spores and vegetative stages were detected in the ovaries of females and testes of males. Infection was also systemic and extensive in larvae of the lepidopteran *Heliothis zea* (Boddie); however, extensive cellular infiltration, nodule formation, and melanization often marked sites of the infections in the fat body and other tissues of this alternate host species.

Host range. Highly susceptible hosts included larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), the false potato beetle, *L. juncta* (Germar), the corn earworm, *H. zea,* the tobacco budworm, *H. virescens* (F.), and the tobacco hornworm, *Manduca sexta* (L.). Larvae and adults of the squash beetle, *Epilachna borealis* (F.), were moderately susceptible while adults of the bean leaf beetle, *C. trifurcata,* were only slightly susceptible. The adult coccinellid, *Coleomegilla maculata* DeGeer, was refractive to infection.

Nosema varivestis n. sp.

Light microscopy. Meronts were mostly spherical and occurred exclusively as cells with a single diplokaryon or as cells in various stages of binary fission (Fig. 22). No stages were seen with more than two diplokarya and all tetranucleate stages represented diplokaryotic cells undergoing binary fission.

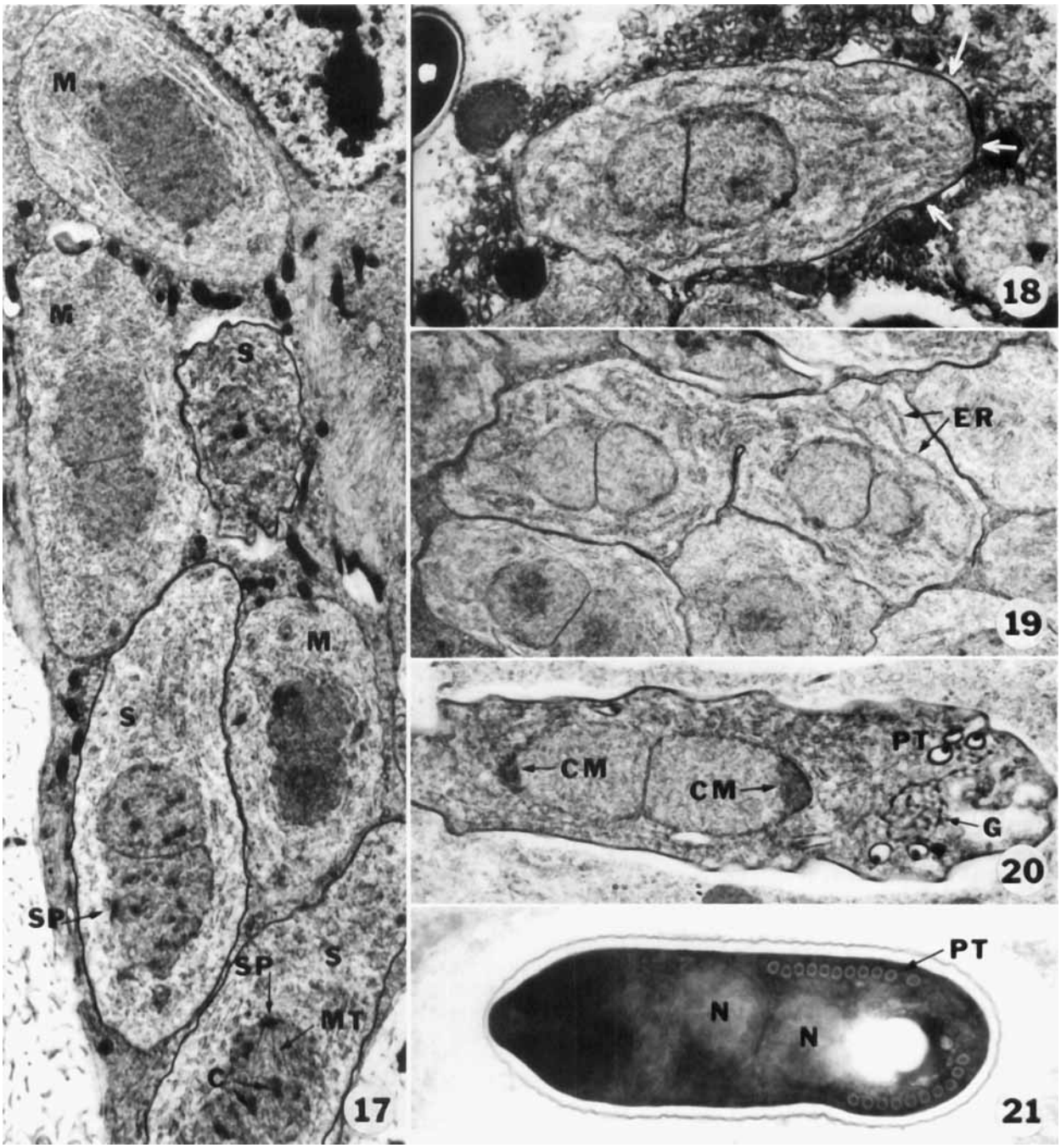
Sporonts stained as intensely with Giemsa as the meronts and were only distinguishable from meronts by their elongate, fusiform shape (Fig. 23); however, the cytoplasm of sporonts undergoing division was usually vacuolated (Fig. 24). Each sporont divided at least once to produce two individual sporoblasts. Late-stage sporoblasts (Fig. 25) were ellipsoidal, diplokaryotic, and highly vacuolated. In some preparations, the diplokaryotic condition of the mature spores was obvious but the interior of most spores was stained too intensely to reveal their nuclear condition (Fig. 26). Fresh spores (Fig. 27) were ellipsoidal to slightly pyriform and measured $4.7 \pm 0.06 \times 2.6 \pm 0.03$ μm (4). Polar tubes, caused to extrude by mixing spores in aqueous solution with an antibiotic mixture of penicillin, fungizone, and streptomycin, had a mean length of 101 μm.

In LAO preparations spherical meronts were observed less frequently, and the elongate, fusiform appearance of most of the vegetative stages made it impossible to distinguish meronts from sporonts. Nuclei, however, were always paired as diplokarya, and the diplokaryotic nature of the sporoblasts was readily confirmed in such preparations (Fig. 27). There was no evidence of karyogamy or meiosis.

Electron microscopy. Meronts (Fig. 28) were elongate and contained a diplokaryon, flattened cisternae of RER, one to few zones of Golgi, and abundant ribosomes. The plasma membrane had a distinct and unusual surface similar to that described for meronts of *Nosema algerae* by Vávra & Undeen (39) and Canning & Sinden (7). The usually indistinct plasmalemma was coated with a thin layer of electron-dense material, the outer surface of which was interspersed with small, regularly arranged tubular structures which encircled the meront transversely (Fig. 28, inset). In longitudinal sections this surface appeared ridged due to the thin lines of the electron-dense material (the exospore) alternating with the parallel electron-transparent areas of the tubules.

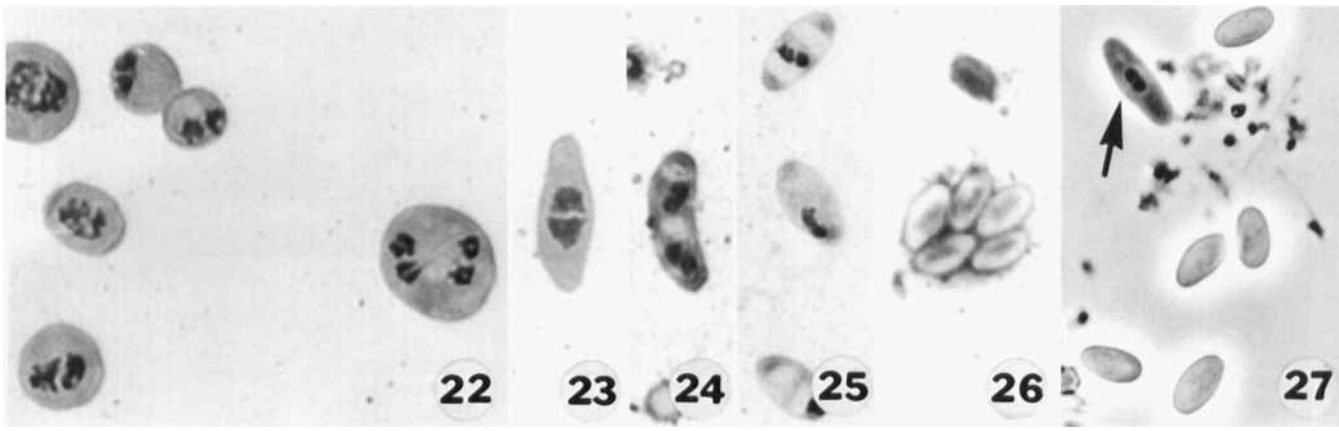
In stages believed to be sporonts (Fig. 29), the tubules were less apparent and they became progressively more obscured as the exospore gradually thickened. The gradual nature of this process, however, prevented a definitive identification of most stages as either meronts or sporonts. Except for differences in the surface coat, sporonts were similar to meronts and both occurred in direct contact with the cytoplasm of the host cell. All stages undergoing binary fission were characterized by deeply invaginated and electron-dense spindle plaques on the surface of the nuclear membranes (Fig. 29). Distinct nucleoli were seldom present but areas apparently representing chromosomes were usually present in nuclei undergoing division. Spindle microtubules were also usually distinguishable in such nuclei.

Unlike most species of microsporidia in the genus *Nosema,* the intracytoplasmic development of this species was marked by the conspicuous appearance of vesicular "granules" in the host cell (Fig. 30). These granules appeared to be secreted from the surface of sporoblasts but may also have been produced by meronts or sporonts. They were decidedly more abundant, however, in cells that contained sporoblasts and spores rather than those with vegetative stages only. Vesicles in the cytoplasm of early stage sporoblasts (Fig. 31) contained electron-dense ma-



Figs. 17–21. Electron photomicrographs of the developmental stages of *Nosema epilachnae*. **17**. Meronts (M) and sporonts (S) in muscle tissue. ×9900. SP, spindle plaques; MT, microtubules; C, chromosomes. **18**. Meront forming a sporont. Note the electron-dense coat (arrows) forming on the right end of the cell. ×10,100. **19**. Dividing sporont with prominent ribosomes attached to lamellae of the endoplasmic reticulum (ER). ×10,000. **20**. Sporoblast with crenated outline. Note the dense mass of clumped material (CM) in the polar region of each nucleus and that coils of the polar tube (PT) are being formed by the Golgi apparatus (G). ×17,000 **21**. Mature spore. ×21,500. PT, polar tube; N, nucleus.

terial that appeared to be deposited on the surface of the plasmalemma resulting in a gradual thickening of the exospore layer. Excessive material in the process of being secreted from the surface of a late-stage sporoblast is shown in Fig. 32. Each bleb of the exospore material has a small electron-transparent center similar to free granules found in the host cell cytoplasm and appears to be liberated from the surface of the sporoblast in the form of a single granule or as a series of interconnected granules (Fig. 32). These interconnected granules were observed most frequently as long chains in the host cell cytoplasm and were closely associated with the surface of the vegetative stages (Figs. 30, 32). They were also observed as shorter chains that sometimes formed circles (Fig. 30, inset), often enclosing free granules.



Figs. 22–27. Developmental stages of *Nosema varivestis* as seen in Giemsa-stained smears (Figs. 22–26) and LAO-stained smears (Fig. 27). ×2000. **22**. Dividing and non-dividing diplokaryotic meronts. **23**. Sporont. **24**. Sporont undergoing binary fission. **25**. Sporoblasts. **26**. Mature spores. **27**. Fresh spores and a late-stage, diplokaryotic sporoblast (arrow).

A second type of secretion was represented by vesicles that occur as a short chain at the tip of a meront (Fig. 30) or as distinct tubules in the host cell cytoplasm (Fig. 29). They were similar in cross section to the tubules that occur on the surface of the exospore material of meronts. Small groups of vesicles with exospore material also occurred occasionally as a mass which appeared to be secreted from the ends of some meronts (Fig. 28).

In addition to vesicles that contained exospore-like material, the cytoplasm of early stage sporoblasts contained agranular ER lamellae, ribosomes, diplokaryotic nuclei, and a rather elaborate cup-shaped structure (Fig. 31), possibly of Golgi origin, containing electron-dense granules. In a later stage of its morphogenesis, this organelle became enlarged and was associated with a spherical, electron-dense body (Fig. 31, inset), the whole of which is probably involved in the formation of the polar sac, the base of the polar tube, and the polaroplast. A network of electron-dense material representing a more typical Golgi apparatus apparently forms the polar tube in the posterior part of mature sporoblasts (Fig. 32). Immature (Fig. 33) and mature spores (Fig. 34) were characterized by the presence of a diplokaryon, ribosomes, polaroplast, 17–19 turns of the polar tube in the peripheral region of the posterior two-thirds of the spore, a posterior vacuole, and a trilaminar spore wall composed of a thick exospore layer, a thicker endospore, and a very thin plasmalemma.

Histological observations. The low virulence of this microsporidium for the MBB was shown by the relatively light areas of infection found in mature larvae that were infected as a result of transovarian transmission. Except for the midgut epithelium, infection was systemic but significant microsporidian development occurred only in the Malpighian tubules. Infections were generally more intense in adult beetles where the microsporidium also invaded the male and female reproductive tissues; however, despite extensive development in the midgut muscularis as well as the Malpighian tubules, the epithelial cells of the midgut were not invaded.

Host range. This microsporidium was moderately infective for larvae of *L. decemlineata* and adults of *C. trifurcata.* Larvae of *L. juncta* and *H. zea* were only slightly susceptible, and no infections were produced in larvae of *H. virescens* or *M. sexta.*

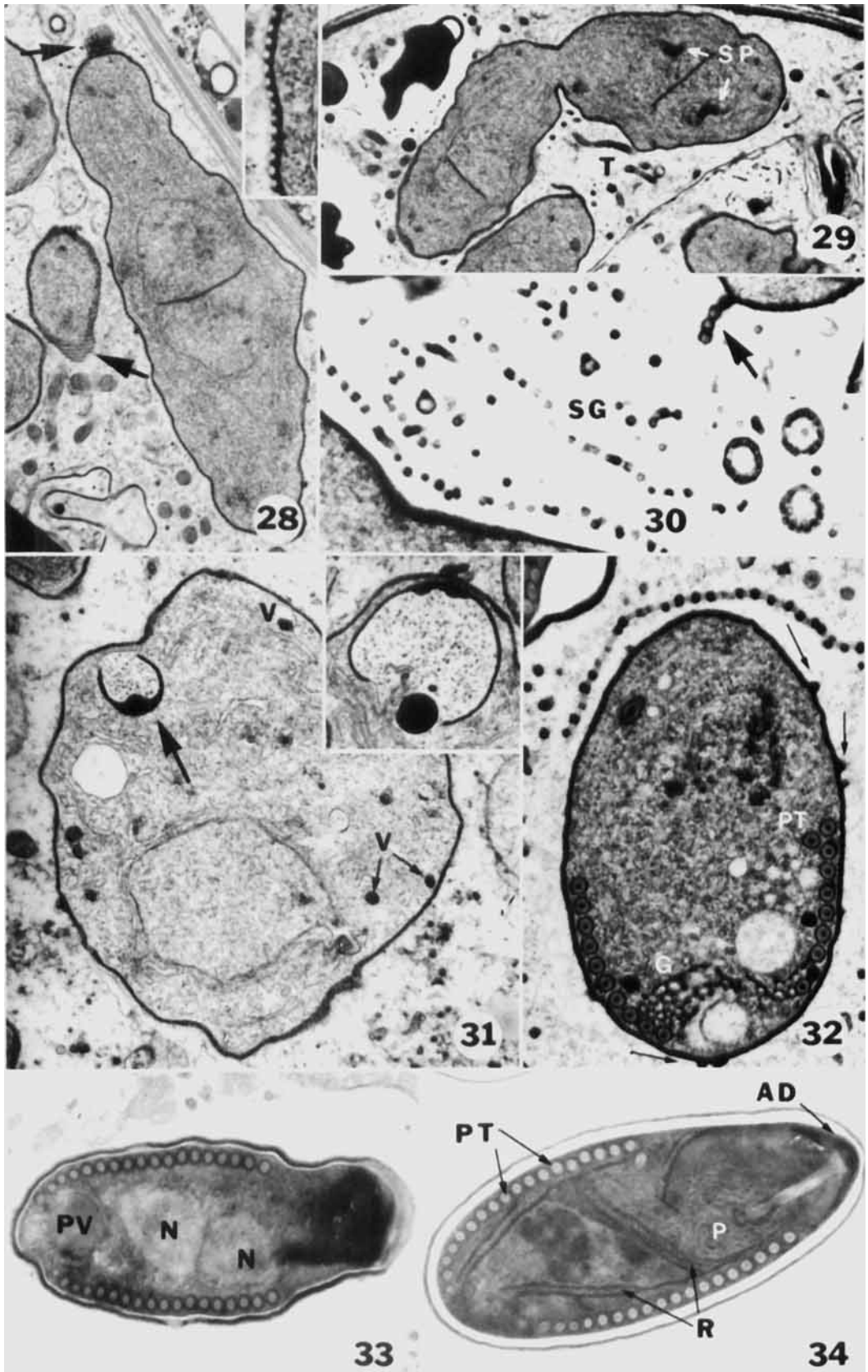
DISCUSSION

Although the order Coleoptera is the largest order of insects, relatively few microsporidia have been described from this host group, most of them belonging to the genus *Nosema.* Thirty-one species and at least eight unnamed and partially described species of *Nosema* have been described from Coleoptera.

The two microsporidian species described herein are also considered to belong to the genus *Nosema,* because they are monomorphic, disporous, and all stages in their life cycles are diplokaryotic. The spores are formed free in the cytoplasm of

→

Figs. 28–34. Electron photomicrographs of the developmental stages of *Nosema varivestis.* **28**. Meront with characteristic plasmalemma. When sectioned longitudinally, the surface is ridged (lower arrow) due to the thin lines of exospore material that alternate with the parallel walls of the tubules. A small mass of tubules and exospore material (upper arrow) is sometimes produced at the tips of meronts. ×9800. *Inset,* enlargement showing the surface features of the meront plasmalemma. ×32,500. **29**. Dividing sporont with thickened layer of exospore material that gradually obscures the encircling tubules on the surface of the plasmalemma. Note the tubules (T) which often occur in the cytoplasm of host cells containing sporoblasts or spores. ×5700. SP, spindle plaques. **30**. Appearance of the secretory granules (SG) in the host cell cytoplasm, which occur as separate granules or in chains. Note small chain of surface tubules (arrow) formed at the tip of a meront. ×18,700. *Inset,* spirals sometimes formed by short chains of the secretory granules. ×15,400. **31**. Early stage sporoblast with a thickened plasmalemma and intracytoplasmic vesicles (V) containing electron-dense material, which is probably deposited on the surface to form the exospore layer of the mature spore. An unusual organelle (arrow), cup-shaped and containing electron-dense granules, also occurs in these early stage sporoblasts. ×11,400. *Inset,* a later stage in the morphogenesis of this organelle, which becomes associated with a dense body. ×11,500. **32**. Mature sporoblast in which coils of the polar tube (PT) are being formed from the Golgi apparatus (G). Note the blebs (arrows) of exospore-like material which are apparently being secreted from the surface to form either single or chains of secretory granules in the host cell cytoplasm. ×16,100. **33**. Immature spore in which the diplokaryon and coils of the polar tube are well demonstrated. ×19,500. N, nucleus; PV, posterior vacuole. **34**. Mature spore. ×19,400. AD, anchoring disc; P, polarplast; R, organized ribosomes; PT, polar tube.


SP
T
29
SG
30
28
V
V
31
PT
G
32
AD
PT
P
R
PV
N
N
33
34

host cells and lack a sporophorous vesicle. Thus, at least these features are shared in common with the type species, *Nosema bombycis* Naegeli, 1857, even though this genus as a whole is very heterogeneous (33, 34). In fact, many of the species of *Nosema* from Coleoptera, as well as from other host groups, have not been shown to possess all of these characteristics and their differentiation as distinct species or as members of the genus *Nosema* is somewhat questionable.

In delimiting species of microsporidia, we are still highly dependent on the old principle of ordinal specificity. Streett et al. (35) provided a summary of the characters of species of *Nosema* known from Coleoptera as of 1975. A similar summary of those species, described in this genus from Coleoptera since their published account, is provided in Table I. As the two species described herein were found to occur naturally in *E. varivestis* and are also infectious for a few species of chrysomelid beetles, emphasis is placed on those species of *Nosema* described from the Coccinellidae and Chrysomelidae. Although both species were also shown to be infectious for a few Lepidoptera, their potential conspecificity with lepidophilic microsporidia is considered remote.

Only four species of *Nosema* have been described previously from coccinellids: *N. coccinellae* Lipa, 1968, *N. hippodamiae* Lipa & Steinhaus, 1959, *N. tracheophilum* Cali & Briggs, 1967, and *N. henosepilachnae* Toguebaye, & Marchand, 1984. Four species are also known from chrysomelids: *N. gastroideae* Hostounský & Weiser, 1973, *N. leptinotarsae* Lipa, 1968, *N. phyllotreatea* Weiser, 1961, and *N. polygrammae* Hostounský & Weiser, 1975. In addition to the differences in their natural host organisms, all of these species differ in one or more major aspects from the two species described herein. None of them are known to produce meronts in chains as is characteristic of *N. epilachnae*, and they all differ from *N. varivestis* in spore shape, size, or host tissue specificity. Except for *N. henosepilachnae*, none of these earlier described species has been examined ultrastructurally; and it is not possible to compare their fine structural features. The most recently described species, *N. henosepilachnae*, differs ultrastructurally from *N. epilachnae* and *N. varivestis* most prominently in the number of turns of the polar tube within the spore.

Although both microsporidia occur naturally in *E. varivestis*, differences in their morphological features and merogonic development indicate their status as distinct species. Meronts of *N. epilachnae* frequently form chains with up to eight nuclei, while *N. varivestis* has only binucleated stages which divide by simple binary fission. Sporoblasts and spores of each species differ in shape and size and in the number of turns of the polar tubes. There are distinct differences in the nature of the plasmalemma and in *N. varivestis* secretory granules and tubules (Fig. 30) can be found in the cytoplasm of infected host cells. There is also an elaborate structure (Fig. 31), possibly of Golgi origin, in sporoblasts of *N. varivestis* that appears to be involved in polar filament-polaroplast differentiation. While infections produced by both species are generally systemic, *N. epilachnae* is decidedly more virulent for the MBB and also infects the midgut epithelium.

The formation of meronts in chains as exhibited by *N. epilachnae* is not typical of most species of *Nosema*. Merogonic chains, however, are formed by *Nosema apis* Zander, 1909, which infects the honey bee *Apis mellifera* L. (12), and by *N. lepocreadii* Canning & Olson, 1980 (6, 8). Thus, this feature would not appear to affect its assignment to the genus *Nosema*. The fine structure of the various stages of *N. epilachnae* are quite typical of other *Nosema* species. The only unusual feature observed was the occurrence of clumped material in the polar region of each of the two sporoblast nuclei (Fig. 20). This feature was consistently present in well fixed sporoblasts but its significance, if any, is unknown.

The ultrastructural features of *N. varivestis* are much more interesting and deserving of further study. The plasmalemma of meronts and sporonts is similar to that described for *N. algerae* (3, 7, 39). A thin layer of electron-dense material overlays the plasmalemma of meronts upon which are situated closely packed tubules that encircle the body transversely. In both *N. algerae* and *N. varivestis*, the electron-dense material appears to be exospore material that eventually forms the outer layer of the spore wall. The gradual deposition of this material on the surface of the vegetative stages eventually obscures the tubules and prevents the ultrastructural identification of most stages as either meronts or sporonts. One can distinguish early meronts from sporonts just prior to sporoblast formation but the intermediate stages differ only in the degree to which the exospore material obscures the encircling tubules. Vávra & Undeen (39) described similar surface structures on the vegetative stages of *N. algerae* but considered all stages they observed at both the light and electron microscopic level to be sporonts. The differentiation of meronts from sporonts in this species, based on the readily visible tubules on the surface of meronts, was initially shown by Canning & Sinden (7) and confirmed by Avery & Anthony (3). In the latter study the tubules were referred to as fibrous protrusions on the limiting membrane, which gradually coalesced to produce a thick-pitted plasmalemma. However, the figures of sporoplasms and early stage meronts show a strong structural similarity to the surface features of meronts described by Canning & Sinden (7) for *N. algerae* and those described herein for *N. varivestis*.

Another interesting and somewhat unusual feature for a species of *Nosema* was the deposition of tubular structures and secretory-like granules by sporoblasts and possibly by other stages of *N. varivestis*. As discussed by Takvorian & Cali (36), various types of tubular appendages and secretory granules are known to be associated with the sporogonic stages of a number of microsporidia of different genera. They described three types of appendages associated with the sporoblasts of *Glugea stephani* (Hagenmüler, 1899) and designated them as types I, II, and III. The tubules produced by *N. varivestis* are somewhat similar to these three types but are not identical to any of them.

Among *Nosema* species that have been examined ultrastructurally, tubules or granules associated with vegetative stages have been described from *N. apis* (42), and *N. algerae* (3, 7), and *N. henosepilachnae* (37). In *N. varivestis* the vesicular chains of tubules occurring on the plasmalemma of meronts (Fig. 30) and the tubules found intracytoplasmically near meronts and sporonts (Fig. 29) are similar to those observed in *N. algerae* (7). They are apparently formed from the tubular material which rests upon the thin layer of exospore material deposited on the surface of the plasmalemma. In *N. varivestis*, exospore material and groups of vesicular tubules are sometimes formed at the distal ends of meronts (Fig. 28). These appear similar to the type II tubules (36). The most interesting secretions in *N. varivestis* are those which appear to be formed primarily from the surface of sporoblasts and occur as single granules or as a series of interconnected granules (Figs. 30, 32). Their formation appears to be related to the secretion of exospore material, and the tubules may be responsible for their occurrence as long chains or small circles (Fig. 30, inset). The tubular material appears to break down rapidly to leave the more electron-dense granules of putative exospore material free in the host cell cytoplasm. These latter types of secretory granules are somewhat similar to type III tubules but appear to be formed differently. Further study of these tubules and secretory granules is needed to establish their exact morphogenesis and similarity to the

TABLE I. *Summary of characters of* Nosema *species described from the Coleoptera since the report of Streett et al. (35).*

Species	Spore shape	Spore size (μm)	Polar filament length (μm)	Principal infected tissues	Host species	Host family
N. adjuncta Hostounský & Weiser, 1980 (18)	Ovoid	4–4.8 × 1.5–1.8 (4.2 × 1.8 average)	No data	Ovaries	*Otiorrhynchus equestris*	Curculionidae
N. albanica Purrini, 1976 (25)	Oval	4–4.7 × 1.6–1.8	No data	Fat body	*Gracilia albanica*	Cerambycidae
N. calcarati Purrini & Halperin, 1982 (28)	Oval	3.5–5.0 × 2.5–3.0	No data	Ovaries and testes	*Pityogenes calcaratus*	Scolytidae
N. costelytrae Hall, Oliver & Given, 1977 (14)	Ovular	2.7–5.0 × 2.0–3.0 (3.3 × 2.3 average)	No data	Fat body	*Costelytra zealandica*	Scarabaeidae
N. dryocoetesi Purrini & Ormières, 1981 (29)	Long, oval	2.5–3.0 × 1.2–1.5	No data	Fat body, lymphocytes, and oenocytes	*Dryocoetes autographus*	Scolytidae
N. epilachnae	Straight to slightly curved, ovocylindrical	5.3 ± 0.13 × 2.1 ± 0.03	75–92 $\bar{x}$ = 84	Fat body, muscles, Malpighian tubules, and most other tissues	*Epilachna varivestis*	Coccinellidae
N. equestris Hostounský & Weiser, 1980 (18)	Widely ovoid	4–5 × 3	No data	Connective tissue, Malpighian tubules, oenocytes, and fat body	*Otiorrhynchus equestris*	Curculionidae
N. gastroideae Hostounský & Weiser, 1973 (16)	Broad oval	3.0–4.8 × 2.5–3 (3.8 × 2.8 average)	25–30	Malpighian tubules and most other tissues	*Gastroidea polygoni*	Chrysomelidae
N. henosepilachnae Toguebaye & Marchand, 1984 (37)	Fusiform	5.4–6.5 × 3.2–4.2	No data	Digestive tube, Malpighian tubules, tracheal cells, muscles, hypodermis, fat body, and reproductive tissues	*Henosepilachna elaterii*	Coccinellidae
N. hylobii Purrini, 1981 (26)	Round or long oval	4.5–6.0 × 1.5–3, macrospores 7.0–8.0 × 2.0–3.5	No data	Midgut	*Hylobius abietis*	Curculionidae
N. hyperae Youssef, 1974 (41)	Ovoidal	3.1 ± 0.15 × 1.7 ± 0.05	40–50	Malpighian tubules, tracheal cells, nerve cord, muscles, epidermal cells, and gonads	*Hypera postica*	Curculionidae
N. polygrammae Hostounský & Weiser, 1975 (17)	Broad oval	4.8 × 2.05	No data	Midgut	*Polygramma undecimlineata*	Chrysomelidae
N. ptinidorum Purrini, 1983 (27)	Not indicated	Highly variable, most were 5.4 × 2.4	No data	Fat body	*Ptinus brunneus*	Ptinidae
N. takapauensis Hall, Oliver & Given, 1976 (13)	Ovular to ovocylindrical	4.5–7.8 × 2.6–3.9 (6.3 × 3.0 average)	No data	Fat body	*Costelytra zealandica*	Scarabaeidae
N. tenebrionides Purrini, 1976 (25)	Not indicated	4.5 × 2–2.5	No data	Fat body (?)	*Tenebrionides mauretanicus*	Tenebrionidae
N. varivestis	Ovoidal to slightly pyriform	4.7 ± 0.06 × 2.6 ± 0.03	87–120 $\bar{x}$ = 101	Malpighian tubules and most other tissues except for midgut	*Epilachna varivestis*	Coccinellidae

tubules and secretory granules formed by other microsporidia. The apparent formation of blebs of exospore material or the possibility that the secretory granules in chains are formed by delamination as a sheath from the surface of the sporoblast needs to be investigated.

The organelle that perhaps needs the most additional study is the elaborate cup-shaped structure (Fig. 31) observed in sporoblasts of *N. varivestis*. A review of papers on spore morphogenesis did not reveal any previously described exact counterpart. Jensen & Wellings (21) described a dense body in sporoblasts

of *G. stephani,* which they suggested was involved in the formation of the anterior part of the polar tube. A similar dense body associated with a vacuole was described by Loubès et al. (22) in sporoblasts of *Glugea truttae* Loubès, Maurand & Walzer, 1981. Vinckier (40) also provided a description of the development and role of Golgi-type vesicles in the formation of the polar sac, polaroplast, and polar tube of *Nosemoides vivieri* (Vinckier, Devauchelle & Prensier, 1970) but similar vesicles associated with the sporoblast nuclei were not observed in *N. varivestis.* We were unable to follow the complete fate of this organelle in our electron micrographs or explain the occasional occurrence of two of these organelles in some sporoblasts. We suspect that it is also of Golgi origin and is involved in the formation of the anterior portion of the polar tube and perhaps even in the formation of the anchoring disc and polaroplast. A typical Golgi apparatus was seen in some sporoblasts (Fig. 32), which appeared to be involved in forming the distal coils of the polar tube.

DIAGNOSIS

Nosema epilachnae n. sp.

Host species. Epilachna varivestis (Coleoptera: Coccinellidae).

Host site. Infections are systemic but particularly heavy in the adipose tissue, muscles, and Malpighian tubules of larvae; also invades reproductive tissues of adults.

Merogonial stages. Bi-, tetra-, and octonucleate forms with diplokaryotic nuclei. Forms chains of from four to eight cells as a result of delayed cytokinesis.

Sporogonial stages. Disporous with diplokaryotic nuclei. Nuclei of sporoblast characterized by clumped material at the pole of each nucleus opposite their common plane of apposition.

Spore. Ovocylindrical in shape, straight to slightly curved, binucleate, measuring $5.3 \pm 0.13 \times 2.1 \pm 0.03$ μm. Polar tube with 10–12 turns and averaging 84 μm in length when everted.

Differentiating characters. High virulence for *E. varivestis,* spore size and shape, and common occurrence of diplokaryotic meronts in chains.

Nosema varivestis n. sp.

Host species. Epilachna varivestis (Coleoptera: Coccinellidae).

Host site. Infections are systemic except for midgut epithelium. Malpighian tubules most heavily infected in larvae. Reproductive tissues also invaded in adults.

Merogonic stages. Occur exclusively as diplokaryotic, binucleate meronts which divide by binary fission. Plasmalemma covered with thin deposit of exospore material on which closely packed tubules that encircle the body transversely are situated. Tubules may also be produced from the plasmalemma and occur in the host cell cytoplasm.

Sporogonic stages. Disporous with diplokaryotic nuclei. Plasmalemma covered by thickened layer of exospore material which obscures the encircling tubules. Granules occurring singly or in chains in the host cell cytoplasm, apparently formed from wall of the sporoblasts. Sporoblasts also contain an unusual organelle, probably of Golgi origin, that may be involved in formation of the polar tube, anchoring disc, and polaroplast in the anterior portion of the spore.

Spore. Ellipsoidal to slightly pyriform, binucleate, measuring $4.7 \pm 0.06 \times 2.6 \pm 0.03$ μm. Polar tube with 17–19 turns and averaging 101 μm in length when everted.

Differentiating characters. Low virulence for *E. varivestis,* spore shape and size, plasmalemma of meronts and early stage sporonts with transversely encircling tubules, which lie on a thin deposit of exospore material, tubules and secretory granules free or in chains in the host cell cytoplasm, and an unusual cup-shaped organelle associated with a dense body possibly responsible for the formation of the polar tube and its associated organelles in the anterior portion of the spore.

LITERATURE CITED

1. Adams, J. R., Tompkins, G. J., Wilcox, T. A. & Schroder, R. F. 1979. Two virus-like particles in the Mexican bean beetle, *Epilachna varivestis. J. Invertebr. Pathol.,* **33**: 111–114.

2. Aldred, J. M., Shepard, M. & Holmes, P. T. 1980. A stochastic model of parasitism of the Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae), in soybeans by *Pediobius foveolatus* (Crawford) (Hymenoptera: Eulophidae). *Res. Popul. Ecol.,* **21**: 286–299.

3. Avery, S. W. & Anthony, D. W. 1983. Ultrastructural study of early development of *Nosema algerae* in *Anopheles albimanus. J. Invertebr. Pathol.,* **42**: 87–95.

4. Brooks, W. M., Montross, D. G., Sprenkel, R. K. & Carner, G. 1980. Microsporidioses of coleopterous pests of soybeans. *J. Invertebr. Pathol.,* **35**: 93–95.

5. Cantwell, G. & Cantelo, W. W. 1982. Potential of *Bacillus thuringiensis* as a microbial agent against the Mexican bean beetle. *J. Econ. Entomol.,* **75**: 348–350.

6. Canning, E. U. & Olson, A. C. 1980. *Nosema lepocreadii* sp. n., a parasite of *Lepocreadium manteri* (Digenea: Lepocreadiidae) from the gut of the California grunion, *Leuresthes tenuis. J. Parasitol.,* **66**: 154–159.

7. Canning, E. U. & Sinden, R. E. 1973. Ultrastructural observations on the development of *Nosema algerae* Vávra and Undeen (Microsporida, Nosematidae) in the mosquito *Anopheles stephensi* Liston. *Protistologica,* **9**: 405–415.

8. Canning, E. U., Olson, A. C. & Nicholas, J. P. 1983. The ultrastructure of *Nosema lepocreadii* Canning and Olson, 1979 (Microspora, Nosematidae) and its relevance to the generic diagnosis of *Nosema* Naegeli, 1857. *J. Parasitol.,* **69**: 143–151.

9. Deitz, L. L., Van Duyn, J. W., Bradley, J. R., Rabb, R. L., Brooks, W. M. & Stinner, R. E. 1976. A guide to the identification and biology of soybean arthropods in North Carolina. *N.C. Agr. Exp. St. Tech. Bull.,* **238**: 264 pp.

10. Douglass, J. R. 1933. Hibernation of the Mexican bean beetle in the Estancia Valley. *N.M. J. Agric. Res.,* **46**: 579–605.

11. Eddy, C. O. 1926. The Mexican bean beetle in South Carolina. *J. Econ. Entomol.,* **19**: 239–248.

12. Gray, F. H., Cali, A. & Briggs, J. D. 1969. Intracellular stages in the life cycle of the microsporidian *Nosema apis. J. Invertebr. Pathol.,* **14**: 391–394.

13. Hall, I. M., Oliver, E. H. A. & Given, B. B. 1976. *Nosema takapauensis* n. sp., a microsporidan parasite of larvae of *Costelytra zealandica* (Coleoptera: Scarabaeidae) in New Zealand. *N.Z. J. Zool.,* **3**: 257–260.

14. ——— 1977. *Nosema costelytrae* n. sp., a microsporidan parasite of larvae of *Costelytra zealandica* (Coleoptera: Scarabaeidae) in New Zealand. *N.Z. J. Zool.,* **4**: 1–3.

15. Hazard, E. I. & Brookbank, J. W. 1984. Karyogamy and meiosis in an *Amblyospora* sp. (Microspora) in the mosquito, *Culex salinarius* Coquillett. *J. Invertebr. Pathol.,* **44**: 3–11.

16. Hostounský, Z. & Weiser, J. 1973. *Nosema gastroideae* sp. n. (Nosematidae, Microsporidia) infecting *Gastroidea polygoni* and *Leptinotarsa decemlineata* (Coleoptera, Chrysomelidae). *Acta Entomol. Bohemoslovaca,* **70**: 345–350.

17. ——— 1975. *Nosema polygrammae* sp. n. and *Plistophora fidelis* sp. n. (Microsporidia, Nosematidae) infecting *Polygramma undecimlineata* (Coleoptera: Chrysomelidae) in Cuba. *Věst. Česk. Spol. Zool.,* **39**: 104–110.

18. ——— 1980. A microsporidian infection in *Otiorrhynchus equestris* (Coleoptera, Curculionidae). *Věst. Česk. Spol. Zool.,* **44**: 160–165.

19. Howard, N. F. & English, L. L. 1924. Studies on the Mexican bean beetle in the Southeast. *U.S. Dept. Agr. Bull.,* **1243**: 50 pp.

20. Howard, N. F. & Landis, B. J. 1936. Parasites and predators of the Mexican bean beetle in the United States. *U.S. Dep. Agric. Circ.,* **No. 418**: 12 pp.

21. Jensen, H. M. & Wellings, W. R. 1972. Development of the

polar filament-polaroplast complex in a microsporidian parasite. *J. Protozool.*, **19**: 297–305.

22. Loubès, C., Maurand, J. & Walzer, C. 1981. Développement d'une microsporidie (*Glugea truttae* n. sp.) dans le sac vitellin de l'alevin de la truite *Salmo fario* L.: étude ultrastructurale. *Protistologica,* **17**: 177–184.

23. Mollenhauer, H. H. 1964. Plastic embedding mixtures for use in electron microscopy. *Stain Technol.*, **39**: 111–114.

24. Own, O. S. 1983. Interactions of the hymenopterous parasite *Pediobius foveolatus* (Crawford) with two undescribed microsporidia pathogenic for the Mexican bean beetle, *Epilachna varivestis* Mulsant. Ph.D. thesis, North Carolina State University, Raleigh, 119 pp.

25. Purrini, K. 1976. Zur Kenntnis der Mikrosporidienarten (Sporozoa, Nosematidae) bei Vorratsschadlingen der Ordnung Coleoptera. *Anz. Schädlingskd. Pfdanzenschutz, Umweltschutz,* **49**: 69–70.

26. ——— 1981. *Nosema hylobii* n. sp. (Nosematidae, Microsporida), a new microsporidian parasite of *Hylobius abietis* L. (Curculionidae, Coleoptera). *Z. Angew. Entomol.*, **92**: 1–8.

27. ——— 1983. Über zwei Protozoen-Arten, *Adelina tribolii* Bhatia comb. nov. (Coccidia) und *Nosema ptinidorum* n. sp. (Microsporidia) als Krankheitserreger der vorratsschädlichen Käfer *Ptinus pusillus* Strm. und *P. brunneus* Dft. (Col., Ptinidae). *Z. Angew. Entomol.*, **95**: 477–482.

28. Purrini, K. & Halpern, J. 1982. *Nosema calcarati* n. sp. (Microsporidia), a new parasite of *Pityogenes calcaratus* Eichhoff (Col., Scolytidae). *Z. Angew. Entomol.*, **94**: 87–92.

29. Purrini, K. & Ormières, R. 1981. On three new sporozoan parasites of bark beetles (Scolytidae, Coleoptera). *Z. Angew. Entomol.*, **91**: 67–74.

30. Quattlebaum, E. C. & Carner, G. R. 1980. A new fungal pathogen of the Mexican bean beetle, *Epilachna varivestis. J. Invertebr. Pathol.*, **35**: 320–322.

31. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.*, **17**: 208–212.

32. Schroder, R. F. W. 1981. Biological control of the Mexican bean beetle, *Epilachna varivestis* Mulsant, in the United States, *in Beltsville Symposia in Agricultural Research, Vol. 5. Biological Control in Crop Production,* Allanheld, Osmun, Granada, pp. 351–360.

33. Sprague, V. 1977. Classification and phylogeny of the microsporidia, *in* Bulla, L. A. & Cheng, T. E., eds., *Comparative Pathology Vol. 2. Systematics of the Microsporidia,* Plenum Press, N.Y., pp. 1–30.

34. ——— 1978. Characterization and composition of the genus *Nosema. Misc. Publ. Entomol. Soc. Am.*, **11**: 5–16.

35. Streett, D. A., Sprague, V. & Harman, D. M. 1975. Brief study of microsporidian pathogens in the white pine weevil *Pissodes strobi. Chesapeake Sci.*, **16**: 32–38.

36. Takvorian, P. M. & Cali, A. 1983. Appendages associated with *Glugea stephani,* a microsporidian found in flounder. *J. Protozool.*, **30**: 251–256.

37. Toguebaye, B. S. & Marchand, B. 1984. Étude ultrastructurale des stages de développement et de la mitose sporogonique de *Nosema henosepilachnae* n. sp. (Microsporida, Nosematidae) parasite de *Henosepilachna elaterii* (Rossi, 1794) (Coleoptera, Coccinellidae). *Protistologica,* **20**: 165–179.

38. Turnipseed, S. G. & Kogan, M. 1976. Soybean entomology. *Annu. Rev. Entomol.*, **21**: 247–282.

39. Vávra, J. & Undeen, A. H. 1970. *Nosema algerae* n. sp. (Cnidospora, Microsporida), a pathogen in a laboratory colony of *Anopheles stephensi* Liston (Diptera, Culicidae). *J. Protozool.*, **17**: 240–249.

40. Vinckier, D. 1975. *Nosemoides* gen. n., *N. vivieri* (Vinckier, Devauchelle & Prensier, 1970) comb. nov. (Microsporidie); Etude de la différenciation sporoblastique et genèse des différentes structures de la spore. *J. Protozool.*, **22**: 170–184.

41. Youssef, N. N. 1974. Biology and pathogenicity of the microsporidian *Perezia hyperae* sp. n., a pathogen of the alfalfa weevil, *Hypera postica. J. Invertebr. Pathol.*, **24**: 282–292.

42. Youssef, N. N. & Hammond, D. M. 1971. The fine structure of the developmental stages of the microsporidian *Nosema apis* Zander. *Tissue Cell,* **3**: 283–394.

Received 17 VIII 84; accepted 5 XII 84

J. Protozool., 32(3), 1985, pp. 535–541

A New Pathogen, *Microsporidium itiiti* n. sp. (Microsporida), from the Argentine Stem Weevil, *Listronotus bonariensis* (Coleoptera, Curculionidae)[1]

LOUISE A. MALONE

Department of Scientific and Industrial Research, Entomology Division, Mt. Albert Research Centre, Private Bag, Auckland, New Zealand

ABSTRACT. A new species of microsporidium (phylum Microspora) infecting the Argentine stem weevil, *Listronotus bonariensis* (Kuschel, 1955), is described on the basis of light and electron microscope observations. It has the following characteristics: nuclei always isolated; meronts spherical and sporonts ribbon-shaped, with variable numbers of nuclei; sporogony within a vacuole which is bounded by a thin membrane that usually breaks down before uninucleate spores mature; occasionally parts of the membrane remain so that clusters of variable numbers of spores may be seen in light microscopic preparations. Spores measure 2.5 × 1.4 μm (fresh) and development occurs mainly in the midgut, but also in the epidermis, fat body, muscle, and ovaries.

DURING a search for pathogens of the Argentine stem weevil, *Listronotus bonariensis* (Kuschel, 1955), a serious pest of New Zealand pastures, many adult weevils were found to be infected with a microsporidium with small spores (10). Microsporidia have been reported from the alfalfa weevil, *Hypera postica* (Gyllenhal) (3, 9, 19), the boll weevil, *Anthonomus grandis* Boheman (11), and the white pine weevil, *Pissodes strobi* (Peck) (15, 18) in the United States of America, and in *Otiorrhynchus* sp. in Czechoslovakia (16) and France (5) as well as in *Pissodes piceae* Ill. in the Soviet Union (7). None have been noted from *L. bonariensis* before. Unusual features, such as multinucleate sporonts in the form of long coiled ribbons and mature spores occurring either singly or in clusters of two to twelve spores, suggested that this microsporidium represented a new species and prompted the detailed light and electron microscope study reported here.

MATERIALS AND METHODS

Infected larval and adult weevils were obtained from a field site at Ruakura Agricultural Research Centre, Hamilton, New Zealand.

[1] I wish to thank G. M. Barker and P. J. Addison, of the Ministry of Agriculture and Fisheries, Ruakura Agricultural Research Centre, Hamilton, New Zealand, for providing me with infected weevils.