

***Nosema blissi* sp. n. (Microsporida: Nosematidae), a Pathogen of the Chinch Bug, *Blissus leucopterus hirtus* (Hemiptera: Lygaeidae)**

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Nosema blissi sp. n. is described from the Malpighian tubules of adults of *Blissus leucopterus hirtus*. Spores measured $6.5 \pm 0.3 \times 2.5 \pm 0.1 \mu\text{m}$ in Giemsa-stained preparations. The polar filament lay in 37 to 40 coils, arranged in a single layer in the posterior portion of the spore, and in several layers in the anterior portion.

INTRODUCTION

Microsporidian infections are widespread in insects, and *Nosema* has been discovered in many species. In the Hemiptera, *Nosema* infections have been reported in the water scorpion, *Nepa cinerea* (Poisson, 1928; Lipa, 1966), the ripple bug, *Velia currans* Poisson, 1929), and the bed bug, *Cimex hemipterus* (Shortt and Swaminath, 1924).

A study to determine the incidence of microbial disease in the hairy chinch bug, *Blissus leucopterus hirtus*, was undertaken, and three adults were found to be infected with *Nosema*. The diagnosis of Microsporidia pathogenic to insects is based largely on the identity of the host, the site of infection in the tissues, and the spore morphology. The new host record and microscopical examination of the pathogen indicate that it has not been described previously. A new species, *N. blissi*, is proposed.

MATERIALS AND METHODS

Seven hundred and forty-six adult chinch bugs, *B. l. hirtus*, from Guelph, Ontario, were examined in 1975. These included 593 which had been field-collected, 59 which had died in cultures, and 94 removed from cultures. Field-collected bugs and those removed from cultures were dissected alive

wherever possible, the remainder being frozen for examination at a later date. Infected tissues were fixed and embedded in Epon blocks according to the procedure of Hayat and Giaquinta (1970). Portions of these blocks were prepared for scanning electron microscopy as described by Humphreys et al. (1973) and examined in an ETEC Autoscan microscope at an accelerating voltage of 20 kV. The remainder was sectioned and photographed in a Philips EM300 electron microscope. Also, smears stained with Giemsa's stain were prepared for examination under a light microscope.

RESULTS

Two brachypterous males and one brachypterous female of *B. l. hirtus* were parasitized by *N. blissi*. One male had died in culture, while the remaining bugs had been field-collected. The microsporidian is described from the Malpighian tubules.

Spores, which were the predominating stage, were packed in the host cell (Fig. 1) in direct contact with the cytoplasm. Their mean length and width were 6.5 ± 0.3 and $2.5 \pm 0.1 \mu\text{m}$, respectively, as measured in material stained with Giemsa's stain. No pansporoblastic membranes were observed.

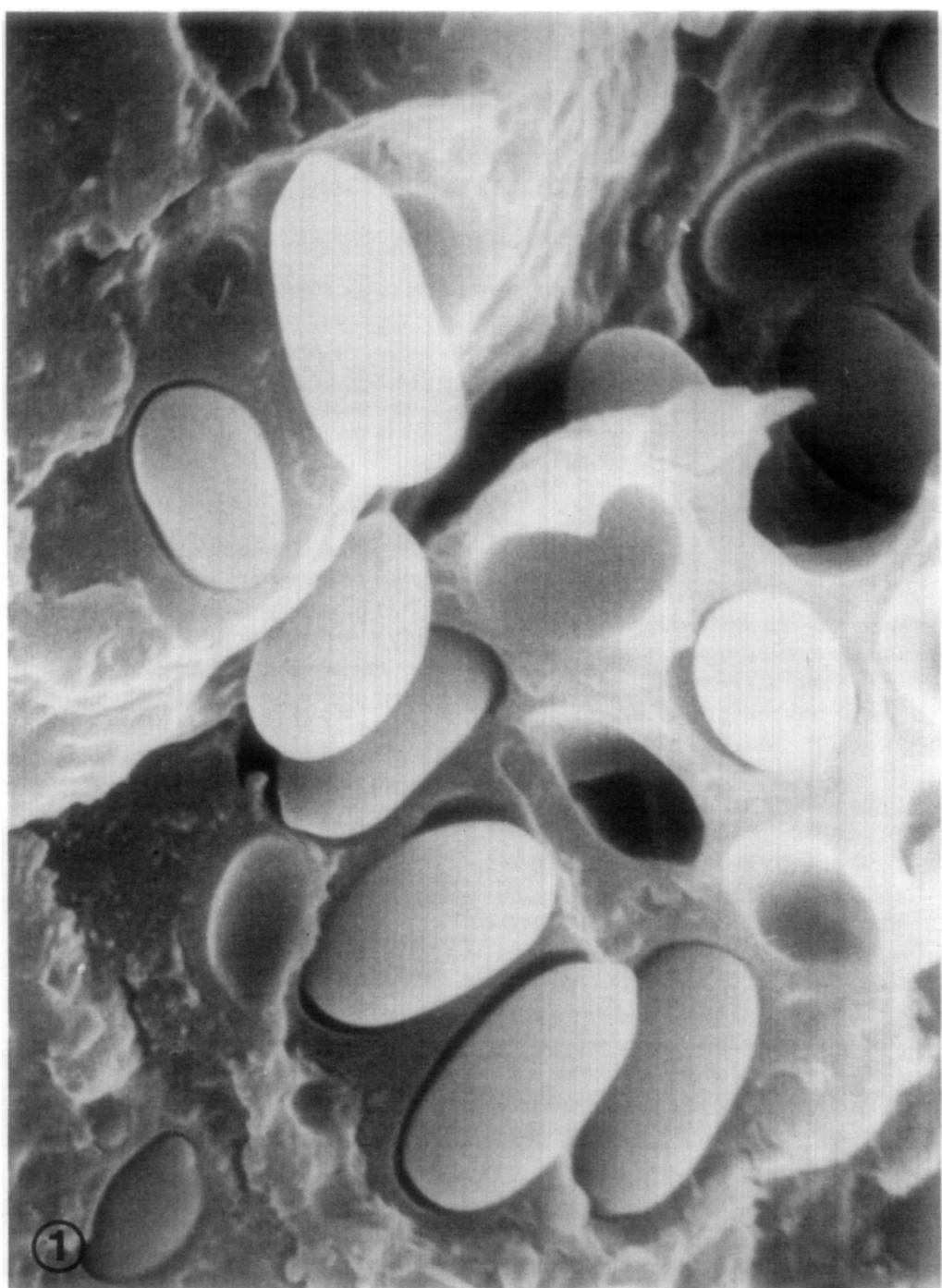


FIG. 1. Spores of *Nosema blissi* in the Malpighian tubules of *Blissus leucopterus hirtus*. Epon block fracture. $\times 8000$.

The polar filament lay in 37 to 40 coils, arranged in a single layer in the posterior portion of the spore, and in several layers in the anterior portion (Fig. 2).

The earliest stage observed was the sporont (Fig. 3). It presented the typical diplokaryon arrangement of the nuclei and was distinguished from a schizont by the

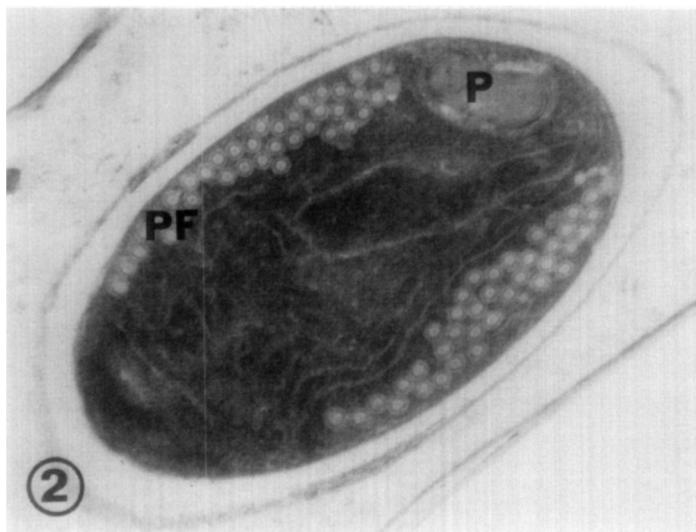


FIG. 2. Spore of *Nosema blissi* from the Malpighian tubules of *Blissus leucopterus hirtus*. The polaroplast (P) is visible at the anterior pole, and the polar filament (PF) possesses several layers of coils in the anterior region. $\times 23,500$.

presence of a wall in addition to the plasma membrane. The schizont possesses a plasma membrane only, as observed by Youssef and Hammond (1971).

Sporoblasts were observed to lie singly in the host cytoplasm and were not surrounded by a pansporoblastic membrane (Figs. 4, 5). The early sporoblast possessed a thicker wall than the sporont and was also recognized by the developing polar filament and the parallel arrangement of the endoplasmic reticulum (Fig. 4). The developed sporoblast (Fig. 5) was characterized by a more electron-dense cytoplasm, a fully-formed polar filament, and the possession of vesicles, which are components of the primitive Golgi complex as proposed by Vavra (1965) and confirmed by Liu and Davies (1972).

DISCUSSION

The pathogen is placed in the genus *Nosema* on the basis of the presence of diplokaryon nuclei and of sporoblasts and spores lying singly in the cytoplasm of the host cell and not enclosed in pansporoblastic membranes. A new species is proposed since *Nosema* has not been reported as a pathogen of Lygaeidae. Weiser (1947)

based the taxonomy of the Microsporidia on the identity of the host, and examination of the literature has indicated that this has been the major consideration in describing species. However, this character has its limitations, since many species of *Nosema* have a wide host range.

At present, it is not possible to diagnose *Nosema* at the species level on the basis of spore size and spore morphology. Overlaps in spore size exist in *Nosema* taken from unrelated hosts in different habitats (Thomson, 1960). However, the spores of *N. blissi* differ from those of *Nosema* infecting other Hemiptera. Both *N. adiei* Shortt and Swaminath and *N. bialoviesiana* Poisson possess spores approximately half the length of those of *N. blissi*. *N. veliae* Poisson possesses curved spores of two size classes, one of which is similar to *N. blissi*. However, all spores of *N. blissi* were of one size and were not curved. Although spores of *N. nepae* Poisson are similar in size to those of *N. blissi*, they have been observed to be very polymorphic.

The external morphology of the spores of several species of *Nosema* that infect insects have been examined by scanning electron microscopy (Lom and Weiser, 1972; Liu and Liu, 1973; Fowler and

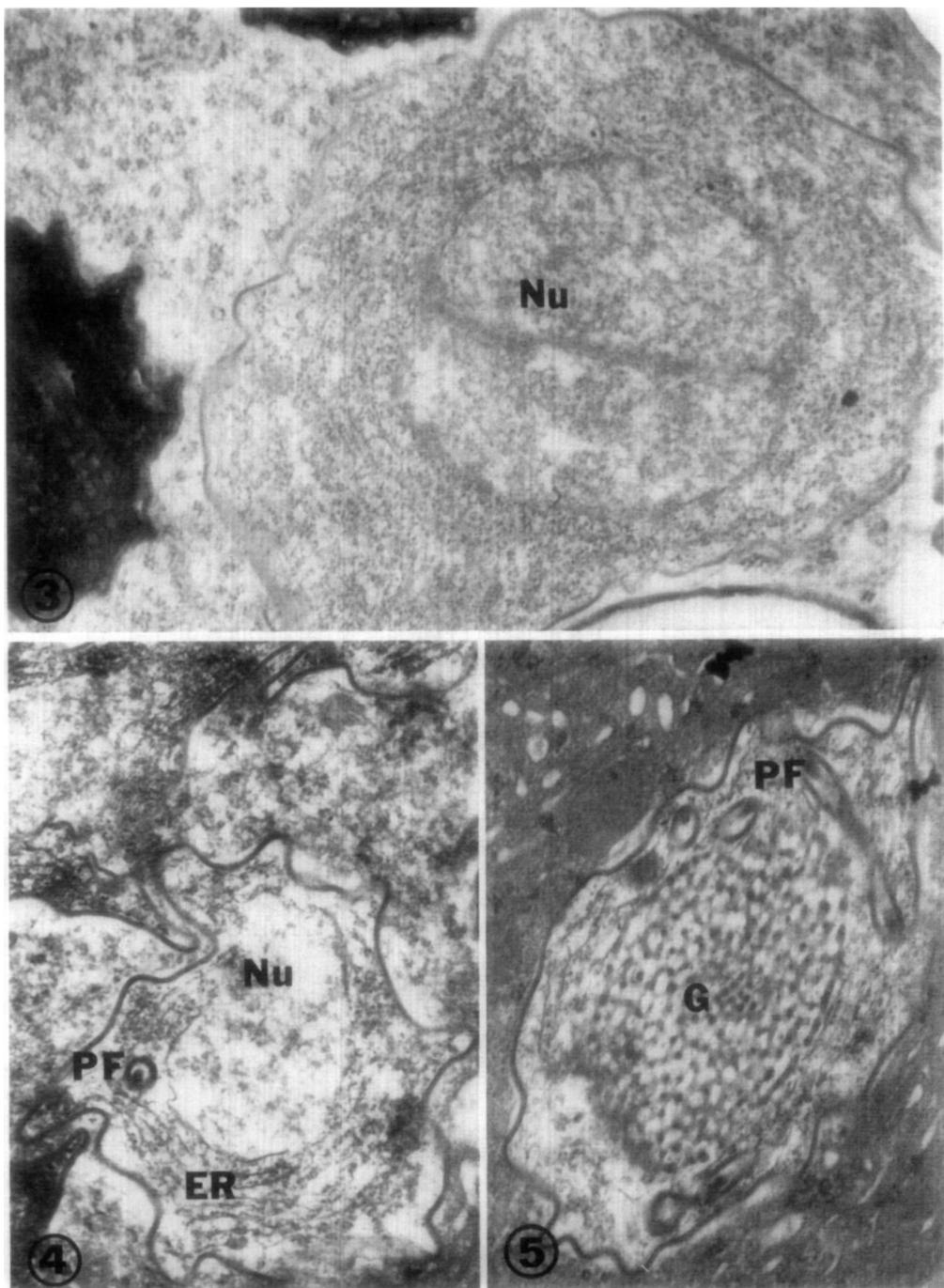


FIG. 3-5. Developmental stages of *Nosema blissi* from the Malpighian tubules of *Blissus leucophaea hirtus*.

FIG. 3. Sporont demonstrating the diplokaryon arrangement of the nucleus (Nu). $\times 15,300$.

FIG. 4. Early-stage sporoblast possessing developing polar filament (PF). The nucleus (Nu) and the parallel arrangement of the endoplasmic reticulum (ER) are observed. $\times 17,060$.

FIG. 5. Developed sporoblast possessing a fully formed polar filament (PF) and primitive Golgi complex (G). $\times 18,600$.

Reeves, 1975). However, different techniques of preparation were utilized, making comparisons at the species level difficult. The method applied in the present study prevents collapse of the spore coat and is identical to that used in a previous study to examine spores of *N. apis*, which were also completely smooth (Liu and Liu, 1973). In addition, it allows the same material to be examined using thin-sectioning techniques.

Since the arrangement of the polar filament has been suggested as a taxonomic character (Burges et al., 1974), the polar filaments of species examined previously using electron microscope techniques, viz. *N. locustae* Canning (Huger, 1960), *N. bombycis* Nageli (Ishihara, 1968), *Nosema* sp. Sprague (Sprague et al., 1968), *N. spelotremae* Guyenot, Naville, and Ponse (Stanier et al., 1968), *N. nelsoni* Sprague (Sprague and Vernick, 1969), *N. apis* Zander (Liu, 1973), *N. whitei* Weiser (Milner, 1972; Burges et al., 1974), and *N. eurytremiae* Canning (Colley et al., 1975) were compared. They were found to differ in the number of coils and their arrangement from the polar filament of *N. blissi*, which was long, lying in 37 to 40 coils. It appears that more widespread application of ultrastructural techniques to studies of *Nosema* promises major contributions to the taxonomy of the genus.

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REFERENCES

- BURGES, H. D., CANNING, E. U., AND HULLS, I. K. 1974. Ultrastructure of *Nosema oryzaephili* and the taxonomic value of the polar filament. *J. Invertebr. Pathol.*, **23**, 135–139.
- COLLEY, F. C., LIE, K. J., ZAMAN, V., AND CANNING, E. U. 1975. Light and electron microscopical study of *Nosema eurytremiae*. *J. Invertebr. Pathol.*, **26**, 11–20.
- FOWLER, J. L., AND REEVES, E. 1975. Microsporidian spore structure as revealed by scanning electron microscopy. *J. Invertebr. Pathol.*, **26**, 1–6.
- HAYAT, M. A., AND GIAQUINTA, R. 1970. Rapid fixation and embedding for electron microscopy. *Tissue Cell*, **2**, 191–195.
- HUGER, A. 1960. Electron microscope study on the cytology of a microsporidian spore by means of ultrathin sectioning. *J. Insect Pathol.*, **2**, 84–105.
- HUMPHREYS, W. J., WODZICKI, T. J., AND PAULIN, J. J. 1973. Fractographic studies of plastic-embedded cells by scanning electron microscopy. *J. Cell Biol.*, **56**, 876–880.
- ISHIHARA, R. 1968. Some observations on the fine structure of sporoplasm discharged from spores of a microsporidian, *Nosema bombycis*. *J. Invertebr. Pathol.*, **12**, 245–258.
- LIPA, J. J. 1966. Miscellaneous observations on protozoan infections of *Nepa cinerea* Linnaeus including descriptions of two previously unknown species of Microsporidia, *Nosema bialoviesiana* sp. n. and *Thelohania nepae* sp. n. *J. Invertebr. Pathol.*, **8**, 158–166.
- LIU, T. P. 1973. The fine structure of the frozen-etched spore of *Nosema apis* Zander. *Tissue Cell*, **5**, 315–322.
- LIU, T. P., AND DAVIES, D. M. 1972. Fine structure of developing spores of *Thelohania bracteata* (Strickland, 1913) (Microsporida, Nosematidae) emphasizing polar-filament formation. *J. Protozool.*, **19**, 461–469.
- LIU, H. J., AND LIU, T. P. 1973. Scanning electron microscope observations on the spore of *Nosema apis* Zander. *Tissue Cell*, **5**, 581–584.
- LOM, J., AND WEISER, J. 1972. Surface pattern of some microsporidian spores as seen in the scanning electron microscope. *Folia Parasitol. (Prague)*, **19**, 359–363.
- MILNER, R. J. 1972. *Nosema whitei*, a microsporidian pathogen of some species of *Tribolium*. II. Ultrastructure. *J. Invertebr. Pathol.*, **19**, 239–247.
- POISSON, R. 1928. Sur une infection à microsporidie chez la Nèpe cendrées (Hémiptera-hétéroptère). La réaction des tissus de l'hôte vis-à-vis du parasite. *Arch. Zool. Exp. Gén.*, **67**, 129–137.
- POISSON, R. 1929. Recherches sur les microsporidies parasites des Hémiptères. III. *Nosema veliae* n. sp., parasite de *Velia currans* Fabr. *Arch. Zool. Exp. Gén.*, **69**, Notes Rev., 53–63.
- SHORTT, H. E., AND SWAMINATH, C. S. 1924. A note on *Nosema adiei*. *Indian J. Med. Res.*, **12**, 181–183.
- SPRAGUE, V., AND VERNICK, S. H. 1969. Light and electron microscope observations on *Nosema nelsoni* Sprague, 1950 (Microsporida, Nosematidae) with particular reference to its Golgi complex. *J. Protozool.*, **16**, 264–271.

- SPRAGUE, V., VERNICK, S. H., AND LLOYD, B. J., JR. 1968. The fine structure of *Nosema* sp. Sprague, 1965 (Microsporida, Nosematidae) with particular reference to stages in sporogony. *J. Invertebr. Pathol.*, **12**, 105–117.
- STANIER, J. E., WOODHOUSE, M. A., AND GRIFFIN, R. L. 1968. The fine structure of the spore of *Nosema spelotremae*, a microsporidian parasite of a *Spelotremia* metacercaria encysted in the crab *Carcinus meanas*. *J. Invertebr. Pathol.*, **12**, 73–82.
- THOMSON, H. M. 1960. A list and brief descrip-
tion of the Microsporidia infecting insects. *J. Insect Pathol.*, **2**, 346–385.
- VÁVRA, J. 1965. Étude au microscope électronique de la morphologie et du développement de quelques microsporidies. *C. R. Acad. Sci. Paris*, **261**, 3467–3470.
- WEISER, J. 1947. Klíč k určování mikrosporidií. *Pr Moravské Přír. Spolectnosti*, **18**, 1–64.
- YOUSSEF, N. N., AND HAMMOND, D. M. 1971. The fine structure of the developmental stages of the microsporidian *Nosema apis* Zander. *Tissue Cell*, **3**, 283–294.