Development of *Nosema partelli* sp. n. (Protozoa: Microsporida: Nosematidae) in the stem borer *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae)

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The development of a new species of *Nosema* (*N. partelli* sp. n.) from the spotted stem borer, *Chilo partellus* (Swinhoe), is described. The microsporidia infect eggs, all larval stages and moths of both sexes of the borer. Infection of the eggs implies that transovarial transmission occurs. Spores of constant size are produced, meronts are mainly binucleated but may contain up to eight nuclei. Presporoblastic meronts are single structures, there are no linear or colony formations. Individual diplokaryotic sporoblasts are chromophilic, and slight overstaining produces almost black spherical and oval structures. The diplokaryotic nucleus is characterized by an achromatic line less than 1 µm in width which divides it through its centre. This characteristic does not occur in other species of *Nosema* and is unique to *N. partelli*. It occurs through all developmental stages from the diplokaryotic sporoblast to the encysting spores. Sporogony is heralded by spindle-shaped diplokaryotic sporoblasts. Diplokaryotic sporonts decrease in size, their nuclei tend to become smaller and pyknotic, after which visible detail is lost following encystment.

Key words: Lepidoptera, *Chilo partellus*, stem borer, Protozoa, Microsporidia, *Nosema partelli*.

INTRODUCTION

The spotted stem borer, *Chilo partellus* (Swinhoe), which was introduced into Africa from India (Mohyuddin & Greathead 1970) is the most destructive pest of maize and grain sorghum in the warm, low altitude regions of southern Africa. It has recently spread and gained economic significance in the Transvaal Highveld (Van Rensburg & Bate 1986). Owing to the inefficacy of insecticidal control, a biological control programme, using exotic parasitoids was initiated. As part of the project, a laboratory culture of *C. partellus* was established from field-collected material to allow for mass rearing and release of the natural enemies. High mortality rates of borer larvae and parasitoids occurred in the cultures, and on closer scrutiny it was observed that all the examined insects were infected by an undescribed microsporidium. These findings led to the study reported here.

Various insect pathogens, including microsporidia, are known to interfere with biological control agents used in weed control (Goeden & Louda 1976). In South Africa the microsporidia *Nosema cactoblastis* and *N. cactorum* were an important factor in retarding progress in the establishment of *Cactoblastis cactorum* (Berg), a pyralid moth that was introduced against prickly pear cacti (Fantham 1939; Petty 1948). *Nosema* decimated the larval population and consequently decreased egg production for several years at the insectaries established for *Cactoblastis* propagation. In the field, the microsporidia also caused high mortality of larvae and pupae (Petty 1948). Reports have also indicated that insect parasitoids are susceptible to microsporidia. The fecundity of the egg parasitoid *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) was reduced by about one half when female parasitoids were infected with *Nosema pyrausta* from their host, the European corn borer, *Ostrinia nubilalis* (Hubner) (Huger 1984).

It is known that microsporidia occur in all vertebrate and invertebrate groups (Canning 1977). *Nosema* sp. was recorded in populations of *C. partellus* from Kenya (Odindo 1991) and from the Comoro Islands (Bordat *et al.* 1984) but, as far as

we know, no microsporidia have been described from *C. partellus* and, furthermore, there is no report of other microsporidia with similar characteristics to those reported here.

MATERIALS AND METHODS

A laboratory culture of the stem borer *C. partellus* was established from field samples collected from maize and grain sorghum plants in the Brits and Warmbaths Districts, Transvaal, South Africa. The insect colony was maintained on an artificial diet (Kfir 1992), at 25 °C and 16:8 (L:D) photoperiod regime. Smears of infected borer eggs, larvae pupae and moths were made on glass slides and fixed in absolute methanol for three minutes, rinsed lightly with tap water, then stained for 30 minutes in 10 % Giemsa's solution and rinsed again. The stained material was examined using a Zeiss compound microscope with photomicrographic equipment fitted with × 60 and × 100 oil immersion objectives. All photomicrographs were printed at × 1000 magnification.

RESULTS AND DISCUSSION

Systematics

Nosema partelli sp. n. (Protozoa: Microsporida: Nosematidae)

Host species: Chilo partellus (Swinhoe) (Lepidoptera: Pyralidae).

Site of infection: fat bodies, malpighian tubules, muscle tissue, alimentary canal, eggs, larvae, pupae and adults.

Localities: Warmbaths and Brits, Transvaal, South Africa.

Sporoplasms: 3 µm in diameter, with indications of diplokaryotic nature.

Meronts: 4 µm in diameter. Nuclei 1 µm in diameter, become multinucleated to contain up to 8 nuclei, 12 µm in diameter.

Merogony: seldom surpasses binucleated stage.

Sporoblasts: diplokaryotic, chromatic separated by achromatic area 1 µm in width. Achromatic area occurs in all subsequent stages. Sporoblasts reach 6–9 µm × 2–3 µm and are tetranucleated.

Spores: 2 × 3 µm and thick-walled.

Etymology: microsporidium which occurs in *C. partellus*.

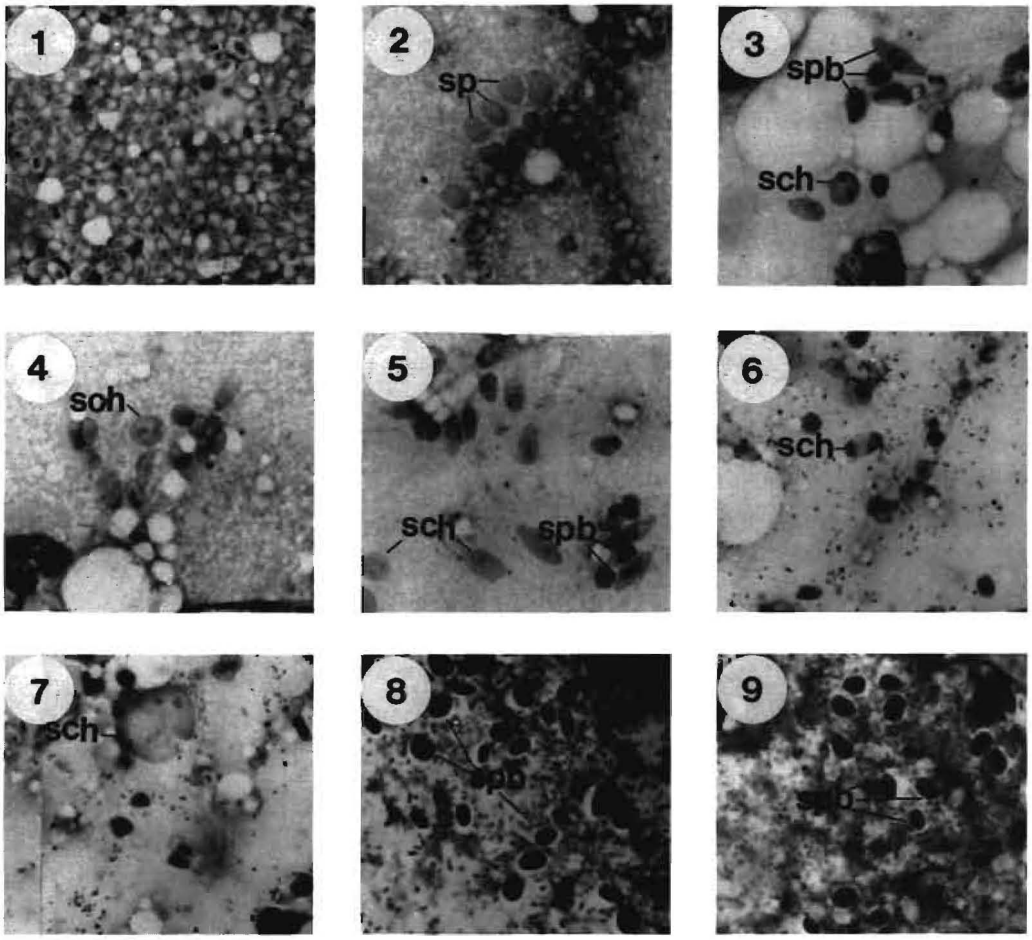
Differentiating characters: the size, appearance and development of sporoplasms, meronts, merogony, sporoblasts and spores.

Type material: the holotype slide of *N. partelli* is deposited in the collection of the Department of Biology, Medical University of Southern Africa (Medunsa). Paratype slides are at the Plant Protection Research Institute, Pretoria.

Description and development: All examined samples from the laboratory culture were observed to be infected with a microsporidium. Mortality rates of up to 100 % were observed when the insects were not maintained under optimal conditions. High larval densities, low relative humidity and faulty preparation of the artificial diet were observed to influence the mortality rate of larvae. *Nosema pyrausta*, a pathogen of the European corn borer, *O. nubilalis*, in the United States of America, causes larval mortality under conditions of environmental stress (Lynch & Lewis 1976; Lewis & Lynch 1976). Under optimal laboratory conditions, mortality rates of *C. partellus* larvae fluctuated between 20 % and 50 %. In a laboratory culture of *C. partellus* in the Comoro Islands an 86 % larval infection by *Nosema* sp. with mortality rates of 40–100 % was reported by Bordat *et al.* (1984).

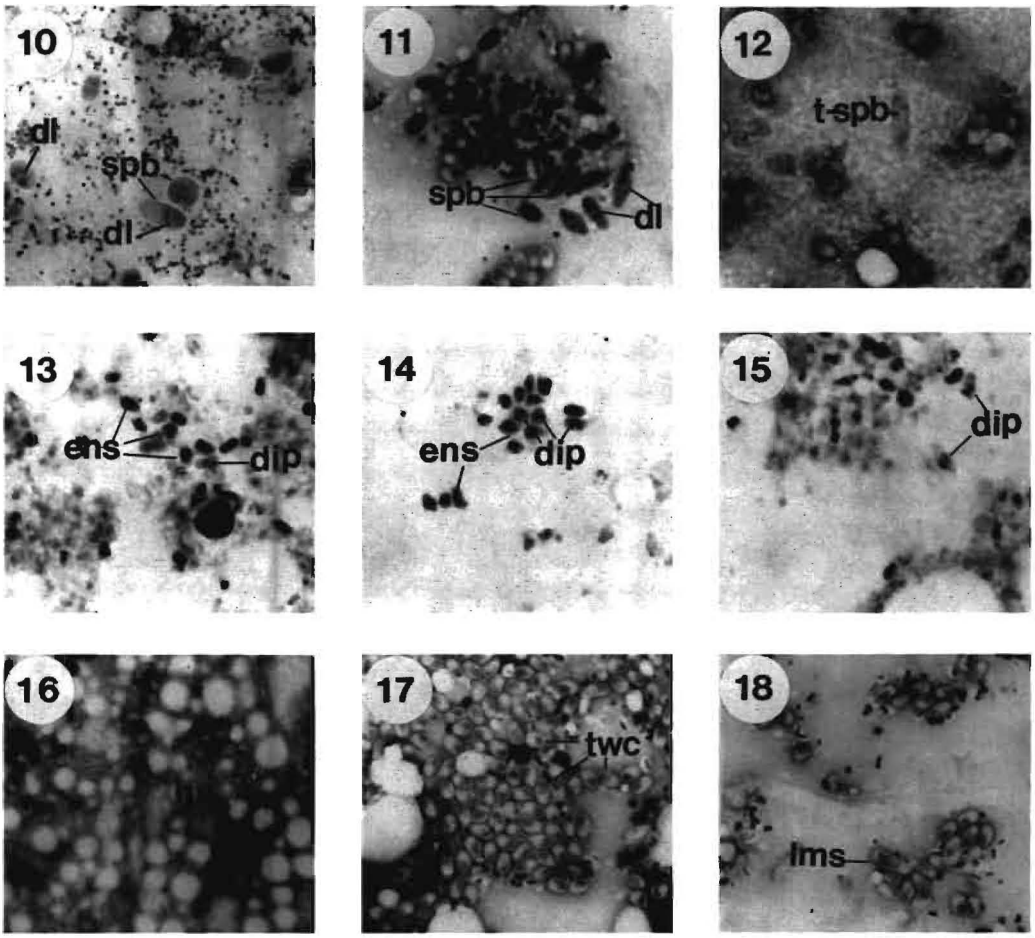
Heavily infected larvae were observed to be lethargic, they refrained from feeding, and their bodies became slightly bloated. The integument lost its transparency due to the accumulation of *Nosema* in the fat bodies under the integument. The microsporidium was also observed infecting the malpighian tubes, muscle tissue and the alimentary canal. In the laboratory culture, some larvae died while others, although heavily infected, completed their development. The surviving insects did not exhibit any signs of distress and no indication of being diseased. It was possible to distinguish diseased from healthy borers only by microscopic examination.

Freshly laid borer eggs contained numerous meronts, sporonts and mature spores in all stages of development, confirming transovarial transmission. This might explain how parasitism reached such high levels in newly hatched larvae. Parasitic development culminates in moths of both sexes harbouring millions of mature spores, and the infection being passed on to the eggs. A specific stage in the life cycle of the parasite would be observed *en mass*, coinciding with disappearance of the preceding stage. This is evident in the



Figs 1–9. 1. Mature spores of *Nosema partelli* sp. n. in a tissue smear from a pupa of *Chilo partellus* (× 1000); 2. Emerged sporoplasms (sp) of *N. partelli* with faint indications of their diplokaryotic nature in a tissue smear from a first instar larva of *C. partellus* (× 1000); 3. A binucleated meront (sch) and sporoblasts (spb) of *N. partelli* in varying stages of development in a tissue smear from a first instar larva of *C. partellus*. Sporoblasts occur as frequently as meronts. Also shown is an enlarged dividing spindle-shaped sporoblast initiating sporogony (× 1000); 4. A binucleated meront (sch) of *N. partelli* with typical bluish-grey mottled cytoplasm in a tissue smear from a first instar larva of *C. partellus* (× 1000); 5. Binucleated meronts (sch) and sporoblasts (spb) of *N. partelli* in different stages of development in a tissue smear from a first instar larva of *C. partellus* (× 1000); 6. A dividing meront (sch) of *N. partelli* in a tissue smear from a pupa of *C. partellus* (× 1000); 7. A rare meront (sch) of *N. partelli* containing eight nuclei in a tissue smear from a pupa of *C. partellus* (× 1000); 8. Spherical overstained sporoblasts (spb) of *N. partelli* in a tissue smear from a pupa of *C. partellus* (× 1000); 9. Oval overstained sporoblasts (spb) of *N. partelli* in a tissue smear from a pupa of *C. partellus* (× 1000).

eggs and young larvae. As the host approaches maturity, all reproduction ceases, giving way to mass production of spores. On reaching maturity, the distended hosts contain millions of mature spores and an absence of merogony and sporogony was observed (Fig. 1). Throughout merogony there is an absence of host-cell vacuoles. By contrast with the sporogonial stages, meronts have a frail appearance with ill-defined nuclei within cytoplasm typified by its mottled bluish-grey colour. This characteristic is used to distinguish these structures from sporoblasts in which



Figs 10–18. 10. Developing and enlarging sporoblasts (spb) of *Nosema partelli* sp. n., approaching encystment stage, with their typical dividing straight line (dl) through the centre of the chromatin forming the diplokaryon in a tissue smear from a pupa of *Chilo partellus* (× 1000); 11. Enlarged spindle-shaped sporoblasts (spb) of *N. partelli* in a tissue smear from a pupa of *C. partellus*. The typical dividing straight line (dl) through the centre of the chromatin is evident (× 1000); 12. A tetranucleated sporoblast (t-spb) of *N. partelli* in an advanced stage of sporogony in a tissue smear from a first instar larva of *C. partellus* (× 1000); 13. Encysting sporonts (ens) of *N. partelli* with diplokarya (dip) still evident in a tissue smear from a pupa of *C. partellus* (× 1000); 14. Encysting sporonts (ens) of *N. partelli* in a tissue smear from a pupa of *C. partellus*. The diplokarya (dip), reduced in size, appear as two pyknotic dots (× 1000); 15. Encysting diplokaryotic sporonts (dip) of *N. partelli*, with spore contents becoming achromatic as encystment progresses. In a tissue smear from a pupa of *C. partellus* (× 1000); 16. Young encysted spores of *N. partelli* in a tissue smear from an egg of *C. partellus*. The nuclei (nu) are pyknotic, the rest of the spore contents stain bluish-grey (× 1000); 17. Mature spores of *N. partelli* in thick-walled cysts (twc) masking the chromatin, resulting in the spores staining bluish-grey in a tissue smear from a pupa of *C. partellus* (× 1000); 18. large mature spore (lms) of *N. partelli* in a tissue smear from a pupa of *C. partellus*. The black dots are decay bacteria (× 1000).

the cytoplasm stains evenly and darker-blue. Sporoplasms initiating merogony are approximately 3 μm in diameter. Despite its size, faint indications of the diplokaryotic nature can be observed (Fig. 2). Meronts of about 4 μm in diameter with two nuclei each of about 1 μm in diameter appear in abundance as merogony progresses (Fig. 3). Merogony is seldom observed beyond the binucleated stage (Fig. 4). Sporoblasts make their first appearance at this stage, which facilitates

identification of the organisms by the sequence of development (Fig. 5). Meronts may become multinucleated. As merogony advances (Fig. 6), meronts are produced containing nuclei which vary in number from one to eight. The size of the meronts seldom surpasses 12 μm in diameter. The nuclei occur within typical mottled bluish-grey cytoplasm (Fig. 7).

No sporogonial vesicles are formed during sporogony, and no other form of parasitophorous vacuoles exist. Sporoblasts and encysting sporonts have evenly distributed cytoplasm which stains dark-blue, also making this a differential characteristic. Overstaining of sporoblasts facilitated their identification. These structures are exceptionally chromophilic, with the result that some have the appearance of black spheres, varying from about 3 μm to about 4 μm in diameter (Fig. 8), with some tending to be oval (Fig. 9), probably as a result of growth preceding sporogony. These structures can be easily confused with overstained mature spores, the latter being achromatic.

Sporoblasts are diplokaryotic, having a characteristic nuclear structure. The chromatin is always separated through the centre by an achromatic straight line, about 1 μm in width (Fig. 10). This characteristic does not occur in other species of *Nosema* and is unique to *N. partelli*. It occurs through all developmental stages from the diplokaryotic sporoblast to the encysting spores. Sporoblasts undergoing single sporogonial division enlarge, become oval, almost spindle-shaped, are 6–9 μm × 2–3 μm, and still possess the dividing line (Fig. 11). This is followed by sporogony (Fig. 3) producing tetranucleated sporoblasts (Fig. 12), after which a final division produces diplokaryotic sporonts ready for encystment.

During encystment of sporonts, diplokarya tend to stain intensely red, yet clearly show the divisional line through the chromatin, accompanied by a decrease in size (Figs 13, 14, 15). Sporonts at the point of final encystment lose visible detail, but still exhibit the unique typical diplokaryotic structure (Fig. 16). After this stage the only indication of spore contents is a bluish mass representing the sporoplasm and probably also the filament, the latter staining more intensely along the inner side of the spore due to its coiled nature. The reddish nuclei are no longer visible once enclosed within the cyst.

Mature spores are thick-walled, hence the chromatic nature of their contents. This masks the chromatin, resulting in masses of bluish-grey mature spores of constant size (Fig. 17). They measure about 2 × 3 μm. Only in very rare instances are outsize spores of about 6.0 × 2.5 μm encountered (Fig. 18).

Diplokaryotic sporoblasts and spores of *Nosema* differentiate this genus from others with its single nucleus which is evident during most of its life cycle. This difference was established by light and electron microscopy (Sprague 1977). *Nosema partelli* is thus characterized by tetranucleated sporoblasts and single spores both of which are diplokaryotic. Merogony is not dependent on host cell vacuoles and meronts have diplokarya. Sporogony does exist but sporoblasts are not confined to sporophorous vesicles. Mature spores are not confined to parasitophorous vacuoles. All these characteristics conform to those of the genus *Nosema*. The diplokarya are characteristic for *N. partelli* with the chromatin divided by a thin achromatic almost linear structure through its centre. It occurs from onset of sporoblast development through the stage when the thick-walled mature spore contents become masked and no further detail can be observed.

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