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An Ultrastructural Study of *Nosema locustae* Canning (Microsporidia) from Three Species of Acrididae (Orthoptera)

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Summary. *Nosema locustae* is a pathogen of orthopterans with an unusually wide host range. It is the only microsporidian that has been developed as a microbial control agent. In spite of its practical importance the ultrastructure of *N. locustae* life stages other than spores, has not been studied. The insects used in this study, all in the family Acrididae, were the Migratory locust, *Locusta migratoria migratorioides* (Oedipodinae), the South American locust, *Schistocerca cancellata* (Cyrtacanthacridinae), and the grasshopper *Dichroplus schulzi* (Melanoplinae). All insects were reared routinely in the laboratory. The spores of *N. locustae* used for the experimental peroral inoculations were all of North American origin. Fat body cells were the predominant site of parasite development, though infection of tracheal epithelium cells and haemocytes also occurred. Ultrastructure of meronts, sporonts, sporoblasts and spores is described. The fine morphology of *N. locustae* stages is typical for microsporidians of the genus *Nosema*. Nuclei were always in diplokaryotic arrangement. Transition from meront to sporont was characterized by striking changes in parasite ultrastructure: appearance of electron dense granules (50-100 nm in diameter, presumable RNP complexes) in the nucleoplasm, and stacks of ER cisternae and prominent vacuoles in cytoplasm. The beginning of sporogony was marked by an increase in size of parasite cells due to extensive vacuolization. Tubule-like structures appeared in the host cell cytoplasm during parasite sporogony. Elongated conglomerates of electron dense material were scattered in the host cell and eventually ornamented the outersurface of the parasite membrane, forming an electron dense layer around sporonts. Spores were diplokaryotic, measured 4.95 ± 0.07 x 2.65 ± 0.04 μm (mean ± SE, n=24) on fresh smears and 3.49 ± 0.18 x 1.73 ± 0.04 μm (n = 10) on ultrathin sections, and had electron-dense cytoplasm in which all internal structures typical of microsporidian spores were recognizable. The polaroplast was lamellar, the endospore was 200-300 nm thick, and the exospore was 40-50 nm. The polar filament was isofilar, arranged in 17-18 coils. Our study did not reveal any difference in the morphology of *N. locustae* while developing in the three different hosts.

Key words: *Dichroplus shulzi*, intracellular parasitism, *Locusta migratoria,*locusts, *Nosema, Schistocerca cancellata,* ultrastructure.

INTRODUCTION

Nosema locustae is a pathogen of orthopterans that was developed as a microbial control agent of grasshoppers (Henry and Oma 1981, Johnson 1997, Lockwood *et al.*1999). Its development was in part possible because unlike most other microsporidia, *N. locustae* has an unusually broad host range. Natural or induced susceptibility to *N. locustae* has been recorded for as many as 102 species of Orthoptera (Brooks 1988, Habtewold *et al.* 1995, Lange and de Wysiecki 1996). The first findings of the species, albeit without naming it, belong to Goodwin (1949), who observed the invasion of the fat body of *Locusta migratoria* R. & F. during his studies on pigment metabolism, and to Steinhaus (1951) who found a new *Nosema* in fat bodies of three species of grasshoppers of the genus

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Melanoplus Stol. The formal description and naming of the species was done by Canning (1953, 1962) who studied the host cycle as it was seen in the light microscope. She observed mostly diplokaryotic proliferative and sporogonic stages but also reported early uninucleate bodies. She placed the new species within genus *Nosema* because "the sporont gives rise to a single spore". Comparative studies of nucleotide sequences of 16S rDNA (Baker *et al.* 1994) and RNA polymerase gene (Cheney *et al.* 2001) placed *N. locustae* in a separate branch on phylogenetic trees, apart from the type species, *N. bombycis*, and other species from lepidopteran hosts considered as "true Nosemas". Surprisingly for an organism that reached commercial development, very little is known about the ultrastructure of *N. locustae*. In what was the first detailed transmission electron microscopy study of a microsporidian spore, Huger (1960) provided the only available ultrastructural information on *N. locustae*, but developmental stages were not studied. Subsequently, some authors (Streett and Henry 1993, Cheney *et al.* 2001) mentioned that ultrastructural examinations of *Nosema* species not closely related to "true Nosemas from Lepidoptera" would be of value for a better understanding of phylogenetic and taxonomic relationships. In addition, an improved knowledge of the morphological characters of *N. locustae* at the ultrastructural level would facilitate its distinction from other known and yet undiscovered *Nosema* species of grasshoppers, particularly after introductions in field populations for control purposes. In this paper we present the results of an ultrastructural examination of *N. locustae* as revealed from infections induced in three species of grasshoppers.

MATERIALS AND METHODS

The insects used in this study, all in the family Acrididae, were the Migratory locust, *Locusta migratoria migratorioides* R. & F. (Oedipodinae), the South American locust, *Schistocerca cancellata* (Serville) (Cyrtacanthacridinae), and the grasshopper *Dichroplus schulzi* Bruner (Melanoplinae). In addition to the availability of experimental insects from the established, healthy laboratory colonies, we chose these particular species for our study because *L. m. migratorioides* is the host type species for *N. locustae*, *D. schulzi* belongs to a subfamily, the melanoplines, which is known for its members exhibiting high susceptibility to *N. locustae* (Henry 1969, Henry *et al.* 1973, Bomar *et al.* 1993, Lange and de Wysiecki 1996) and *S. cancellata* is not only a major agricultural pest in Argentina (Hunter and Cosenzo 1990), but is also closely related to the desert locust, *Schistocerca gregaria* (Forskål), a major pest in northern Africa and the Middle East (Steedman 1990). The colony of *L. m. migratorioides* was maintained under controlled conditions (30ºC, 16L : 8D photoperiod, 40-45% RH; diet of wheat seedlings and reed grass) in the insectarium at the Laboratory of Microbiological Control of the All-Russia Institute for Plant Protection, St. Petersburg. The colonies of *S. cancellata* and *D. schulzi* were maintained according to general procedures described by Henry (1985), in rearing rooms (30°C, 14L : 10D, 40% RH; diet of wheat seedlings and bran, lettuce, cabbage) at the Center for Parasitological Studies (CEPAVE) of La Plata National University.

The spores of *N. locustae* used for the experimental inoculations were all of North American origin. Those used for the inoculation of *L. m. migratorioides* were kindly provided by J. E. Henry and D. A. Streett from the United States Department of Agriculture - Agricultural Research Service, Rangeland Insect Laboratory, Bozeman, Montana. The spores employed for the inoculations of *S. cancellata* and *D. schulzi* were isolated and purified by the homogenization procedure (Henry and Oma 1974) from infected grasshoppers [the melanoplines *Dichroplus pratensis* Bruner*, D. elongatus* Giglio-Tos, and *Baeacris punctulatus* (Thunberg)] collected in fields of central Argentina, after *N. locustae* became established in grasshopper communities following its introduction from North America between 1978-1982 (Lange and de Wysiecki 1996, Lange 1999, Lange and Cigliano 1999). The spore concentrates used in the introductions were also produced by J. E. Henry´s group at Montana´s Rangeland Insect Laboratory.

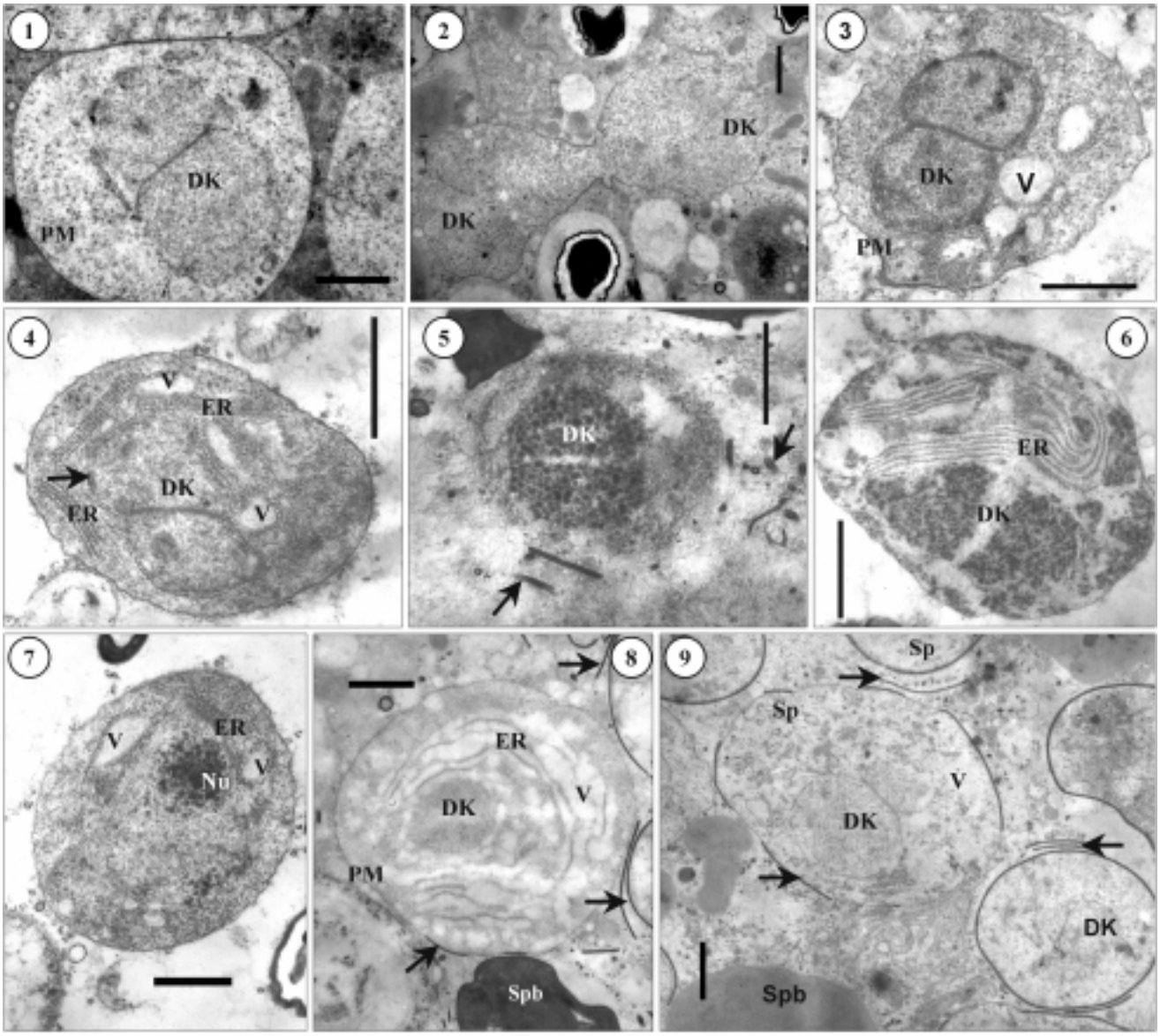
The *per os* standard inoculation protocol utilized to challenge grasshoppers with spore suspensions of microsporidia (Habtewold *et al.* 1995, Lange *et al.* 2000) was used to successfully induce infections in individuals of the three host species employed. Third-instar nymphs ingested 10^5 spores each.

For transmission electron microscopy, following ventral dissection of infected insects small samples of tissues were fixed for 1h 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 Epon-Araldite or Spurr´s resins. Thick sections (1.0 μm) stained with methylene blue were observed under the light microscope, to determine the infected sites. Ultrathin sections were poststained with methanolic uranyl acetate followed by lead citrate, and were observed and photographed at an accelerating voltage of 70 kV with a Hitachi H-300 electron microscope.

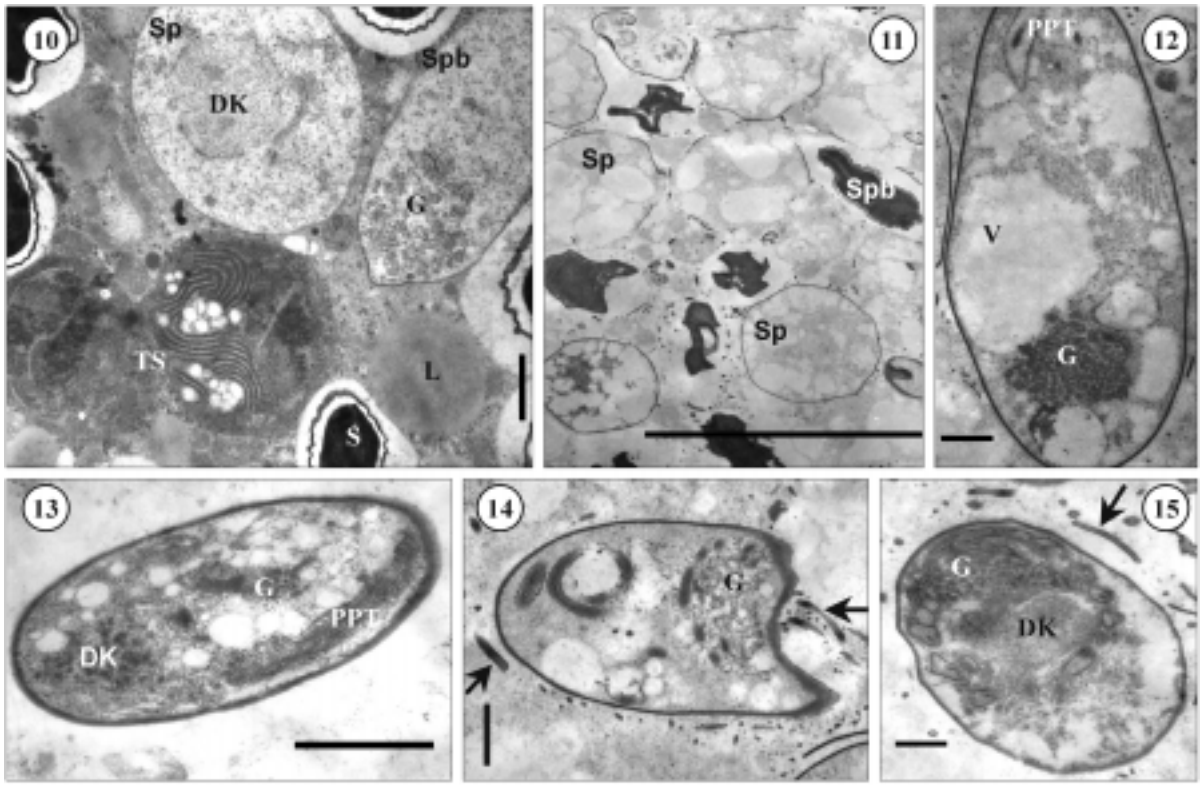
The contribution follows the terminology and conceptual basis proposed for the microsporidia by Sprague *et al.* (1992).

RESULTS

We observed the same kind of stages and the same pattern of development of the pathogen in all three host species studied. The cytoplasm of the insect´s adipose tissue cells was the overwhelmingly predominant site of development of *N. locustae*, although infection was also, albeit infrequently, present in cells of tracheal epithelium. The accumulation of parasites in the cytoplasm caused enlargement and fragility of host cells. As a result,



Figs 1-9. Fine morphology of proliferate stages of *Nosema locustae* development. **1** - meronts in a host adipocyte*;* **2** - binary fission of a tetranucleate meront; **3** - early meront/sporont transitional stage. Note appearance of large vacuoles outside nuclear envelope, electron dense globules inside the nucleus and thickening of the plasma membrane; **4 -** transitional stage. Rough endoplasmic reticulum is arranged in regular multilayer patterns. Note vacuolization of the cytoplasm in the vicinity of ER stacks; **5** - transitional stage. Numerous electron dense particles are scattered throughout the nucleoplasm. Arrows indicate electron dense tubular structures inside host cytoplasm; **6 -** typical characters of the transitional stage: diplokaryon (DK) with intranuclear particles, whorls of endoplasmic reticulum inside the cytoplasm. The electron dense envelope has not started to form yet; **7** - the same stage as on Fig. 6. Vacuoles appear as the expanded lumens of endoplasmic reticulum; electron dense granules are accumulated in nucleolus-like structure; **8** - early sporont. Electron dense layer is deposited on the external surface of its plasma membrane and on adjacent sporonts; similar elongated conglomerates of electron dense material are scattered inside the host cell (arrows). Expanded cisternae of rough endoplasmic reticulum (vacuoles) occupy the most space of the cell; **9** - roundish sporonts: nucleus/cytoplasm ratio is obviously lesser than in previous stages. Arrows indicate ribbons of electron dense material attached to the outer surface of the parasite cell or scattered free inside the host cytoplasm. DK - diplokaryon, ER - endoplasmic reticulum, Nu - nucleolus-like structure, PM - plasma membrane, V - vacuole, Sp - sporont, Spb - sporoblast. Scale bars - 1μm

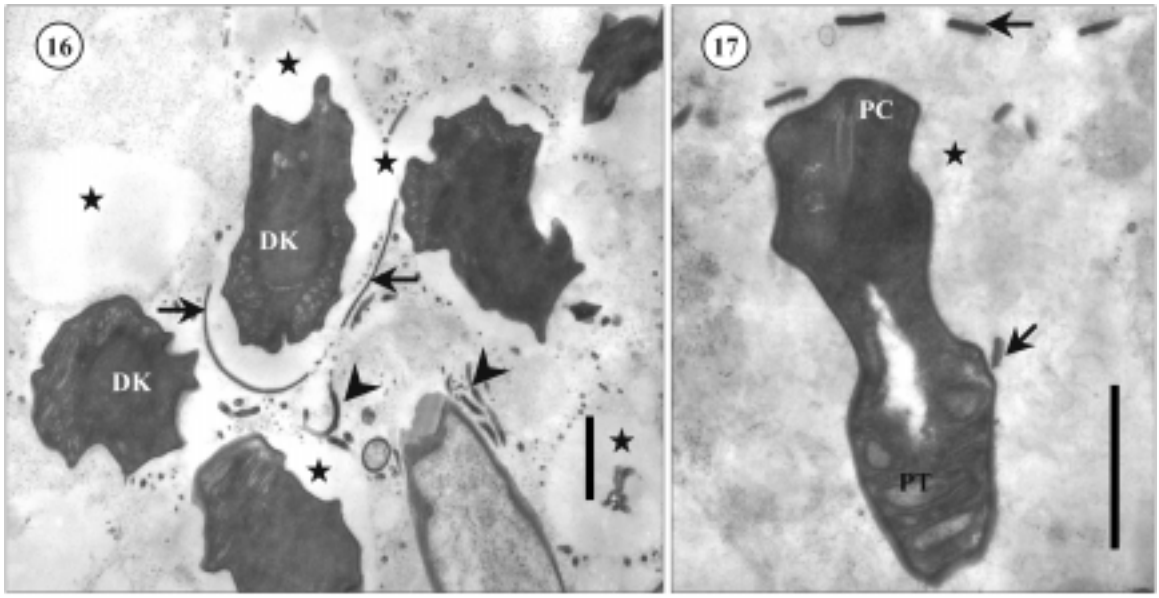


Figs 10-15. Fine morphology of *Nosema locustae* sporogony stages. **10, 11** - sections through the adipocyte, containig various pre-spore stages of *N. locustae*: pair of "transitional" stages; spores; sporonts; sporoblasts; **12** - parasite cell combining sporont (big cell size and prominent vacuolization of the cytoplasm) and sporoblast (cell polarization, thick wall, Golgi cluster and a primordial polar tube) characters; **13 -** oblique section through an early sporoblast with primordial polar tube; **14, 15 -** sporoblasts. Elements of the polar tube mature inside a peripheral region of the vesicular-tubular Golgi cluster. Tubular-like structures (arrows) are abundant in host cell cytoplasm during sporogony. DK - diplokaryon, G - Golgi apparatus (vesicular tubular cluster), L - lipid droplets, PPT - primordial polar tube, S - spore, Sp - sporont, Spb - sporoblast, TS - meront/sporont transitional stage, V - vacuole. Scale bars: Figs 10, 12-15 -1 μm; Fig. 11 - 10 μm

heavily infected fat bodies were hypertrophied and easily disrupted. In some instances, mature spores were seen in membrane bounded compartments (presumably phagosomes) in haemocytes. Our attempts at observing early stages of development in intestinal epithelium at 48-96 h postinoculation failed. All stages of the parasite were in direct contact with the host cell cytoplasm (i.e., no interfacial envelopes were present).

Meronts (Fig. 1), as defined by Sprague *et al.* (1992), were the earliest stage in the life cycle of *N. locustae* that we recognized. They were round or slightly oval in shape, ranged from 3.5 to 5.0 μm in diameter as measured in ultrathin sections. Meronts normally possessed two or four nuclei in diplokaryotic arrangement. Merogony was by binary fission of tetranucleate stages (Fig. 2). Meront ultrastructure was typical of microsporidia (Sprague *et al*. 1992, Becnel and Andreadis 1999). Meronts were surrounded by a simple plasmalemma of 7.5 to 10.0 nm thick. The cytoplasm had numerous free ribosomes and a few membrane profiles (Figs 1, 2). Condensed chromatin was not observed in nuclei of meronts even when they were undergoing binary fission (Fig. 2).

Transitional stages from merogony to sporogony (Figs 3-7, 10) were abundant in adipocytes of all three examined hosts. These intermediate stages were identifiable by the set of morphological features, mentioned beneath, which may be present in various combinations. (i) Appearance of numerous electron dense intranuclear particles scattered throughout the nucleoplasm (Figs 5, 6) or assembled in the nucleolus-like structure (Fig. 7). Sometimes the accumulation of electron dense particles 50-100 nm in diameter made the boundary between two nuclei of the diplokaryon nearly invisible (Fig. 5). The same structures were visible in parasite cytoplasm outside the nucleus (Fig. 6). (ii) Appearance of abundant



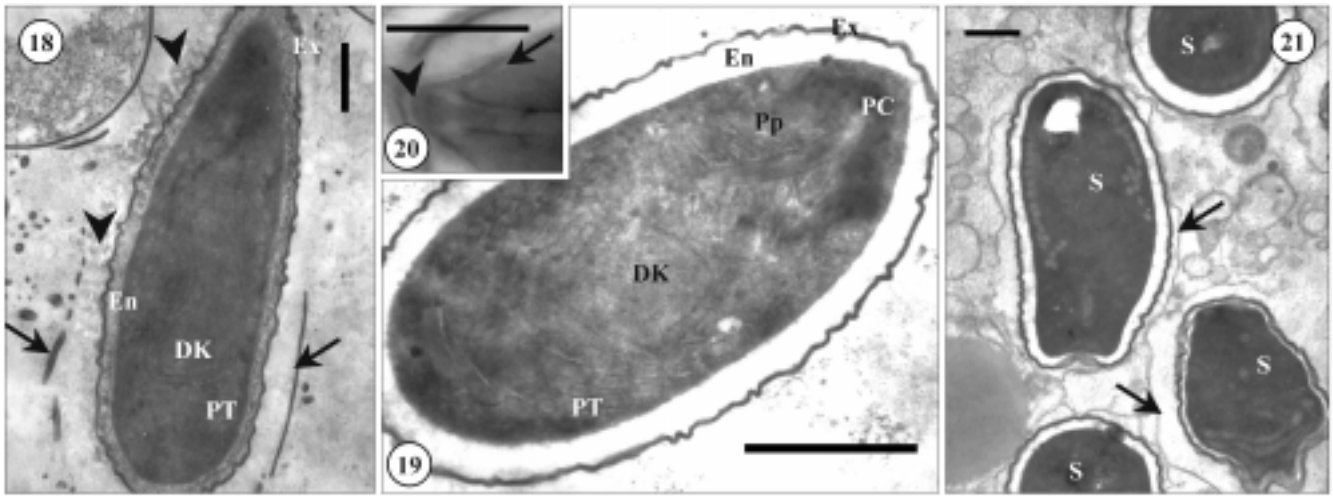
Figs 16, 17. *Nosema locustae* "late" sporoblasts. The shrinkage of the parasite cell during "early"sporoblast/ "late" sporoblast transition leaves behind the electron-lucid space (stars) limited the by the electron dense layer (arrows), which eventually degrades in the host cell cytoplasm. Tubular-like structures (arrowheads), which are usually larger in diameter compared with the above-mentioned electron dense layer, sometimes can hardly be differentiated from the latter. DK - diplokaryon, PC - primordial polar cup, PT - primordial polar tube. Scale bar - 1 μm

rough endoplasmic reticulum often arranged in regular multilayer patterns (Figs 4, 6, 11); (iii) conspicuous vacuolization of the cytoplasm in the vicinity of ER stacks or around the nuclei. (Figs 3, 4, 7). The plasmalemmas of transitional stages were *ca* 10-12 nm thick and showed an undulating profile with numerous evaginations. Small vesicles were normally present in close vicinity. The parasite cells at this stage were round or slightly elongated; their diameter, measured on ultrathin sections, varied from 2.9 to 3.6 μm. Further vacuolization of the cytoplasm lead to an increase of the parasite´s cell volume.

Sporonts (Figs 8-11) were revealed in all studied hosts as round, large cells of 4.8-5.5 μm in diameter, containing one diplokaryon with evidently lesser nuclear/cytoplasm ratio comparatively with the previous stages. Multiplication and development of sporonts were accompanied by emergence of tubule-like structures in the host cell cytoplasm. Elongated conglomerates of electron dense material were scattered inside the host cell and eventually ornamented the external surface of the parasite membrane, forming a continuous dense layer of about 25 nm thick (Figs 8, 9).

Sporoblasts were readily distinguishable from sporonts by their gradual elongation and polarization, and by further thickening of the wall (Figs 12, 13). Polarization was manifested by the appearance of a distinct vesicular-tubular cluster [Golgi-like complex (Cali and Takvorian 1999)] in the posterior part of the cell and the primordial apical part of the polar filament anteriorly (Figs 12-15). The elements of the polar tube appeared to mature inside the peripheral (trans-Golgi) region of the vesicular-tubular cluster (Fig.15). From a certain point during sporogenesis, reduction in size of sporoblasts was evident (star-like stage) (Figs 16, 17). The shrinkage seem to occur abruptly, leaving behind the space formerly occupied by the sporont, recognizable by an electron dense layer, which eventually degraded in the host cell cytoplasm. The shrinkage, as a phenomenon of "extensive vacuolization" observed on the "transitional" stage, represents possibly a fixation artifact, which yet might reflect the alteration in membrane properties and its permeability taking place at these stages. As in earlier developmental stages (Fig. 5), tubular structures continued to be abundant in the host cytoplasm around sporoblasts (Fig. 16). Sporoblast sizes varied considerably: 2.2-3.8 x 1.0-2.8 μm.

Spores were diplokaryotic, measured 4.95 ± 0.07 x 2.65 ± 0.04 (mean ± SE, n=24) in fresh smears and 3.49 ± 0.18 x 1.73 ± 0.04 (n=10) in ultrathin sections and



Figs 18-21. *Nosema locustae* spores. **18** - young spore. The internal structure of the endospore is visible, as well as tubules (arrowheads) filling the zone of host cell cytoplasm between the parasite exospore and former borders of the "mother" sporont marked by the ribbons of electron dense material (arrows); **19** - mature spore with a typical set of organelles; **20 -** section through the apical part of a polar filament, revealing an anchoring disk (arrowhead), a polar sac (arrow) and the anterior part of a polaroplast, composed of tightly packed membranes; **21 -** in locust haemocytes spores were found residing inside membrane bound compartments (arrows), presumably parasitophorous vacuoles. DK - diplokaryon, En - endospore, Ex - exospore, PC - polar cap, Pp - polaroplast, PT - polar tube, S - spore. Scale bars: Figs 18, 19, 21 - 1 μm; Fig. 20 - 0.5 μm

had an electron-dense cytoplasm. Internal structures of the spore - anchoring disc, polar filament, lamellar polaroplast, diplokaryon, layers of endoplasmic reticulum and posterior vacuole (Figs 18-21) showed the characteristic organization of "typical microsporidia" (Vavra and Larsson 1999). The spore envelope was composed of the 200-300 nm thick electron-lucid endospore, which exhibited sometimes a fibrous structure (Fig. 18), and the undulating, electron-dense exospore 50-60 nm thick with no differentiated structure. The polar filament was isofilar, arranged in 17-18 coils. In *Locusta migratoria* haemocytes spores were sometimes enclosed in membrane-bounded compartments, presumably phagosomes (Fig. 20)

DISCUSSION

This is the first study showing the ultrastructure of developmental stages of *N. locustae*. The only information at the electron microscopy level previously available was on spores (Huger 1960). We found justification in including in the present contribution our own observations on spores because although Huger (1960) did not specify the precise origin of the isolate of *N. locustae* he used for his study, he did not work with material of North American origin but with infections in laboratory-reared *L. m. migratorioides*, as Canning (1953) did for the original description of the pathogen. Therefore, since we utilized material of North American origin we could conceivably have found some differences. However, our observations of spores are in good general agreement with those of Huger (1960). The slight discrepancies observed (measurements of endospore and exospore, and presence-absence of undulating profiles of exospore) can conceivably be attributed to the utilization of different fixation protocols. Huger (1960) used much longer fixation times (44 h) than we did (1½ h) and used $OsO_4/K_2Cr_2O_7$ only while we used double fixation (glutaraldehyde/OsO_4). Similarly, the stages observed by us by electron microscopy appeared consistent with those originally reported by Canning (1953, 1962) using light microscopy, not only in size and shape but also in nucleus/cytoplasm ratio. The only essential difference was that we did not observe uninucleate stages, but if we have had observed them it would not have been reliable information because sectioned material is misleading for establishing number of nuclei. Also, a stage like the one depicted in Fig. 5, having the nuclei of the diplokaryon in extremely intimate contact might have been easily recognized as uninucleated in light microscopy. Uninucleate stages have been shown in light microscopy studies of

Table1. *Nosema* species from Orthoptera. A - adipose tissue, B - blood cells, C - gastric caeca, G - gonad, H - haemolymph, M - muscle, Mg - midgut, Mt - Malpighian tubules, N - neural tissue, P - pericardium, S - salivary glands, T - traqueal matrix

Species	Type host (family: subfamily)	Tissue/organ affected	Spore size (fresh) in µm	Nr. polar filament coils	Salient features	References
N. acridophagus	*Schistocerca americana* (Acrididae: Cyrtacanthacridinae)	Mg, G, P, N, A	4.1 x 2.6	10-12	Bi- or tetra nucleate meronts covered by layer of tubular elements	Henry 1967, Streett and Henry 1993
N. asiaticus	*Oedaleus asiaticus* (Acrididae: Oedipodinae)	Mg, Gc, G, Mt, A	4.2 x 1.8	11	Multinucleate meronts (up to 12 nuclei)	Wen 1996
N. chorthippi	*Chorthippus albomarginatus* (Acrididae Gomphocerinae)	A	3.5 x 1.9	?	Meront and sporont plasma membrane is ornamented with electron dense material arranged in "bead-like" structures	Issi and Krylova 1987
N. cuneatum	*Melanoplus confusus* (Acrididae: Melanoplinae)	P, A, G, T, Mg, Mt, N	4.8 x 3.4	10-12	Cuneate spores; globulo-vesicular layer around transitional stages	Henry 1971, Streett and Henry 1987
N. grylli	*Gryllus bimaculatus* (Gryllidae)	A, H	4-5.2 x 1.8-2.5	18-20	Transitional meront/sporont stage with numerous electron dense intranucleus particles	Sokolova *et al.* 1994
N. locustae	*Locusta migratoria* (Acrididae: Oedipodinae)	A	4-6.5 x 2.5-3.5	15-18	Transitional meront/sporont stage with numerous electron dense intranucleus particles	Canning 1953, Sokolova and Lange ibidem
N. maroccanus	*Dociostaurus maroccanus* (Acrididae: Gomphocerinae)	M, Mg	4.4-5 x 2.5-3.8	14-15	No information	Issi and Krylova 1987
N. montanae	*Melanoplus packardii (*Acrididae: Melanoplinae)	A	3.1 x 1.5	5-7	Multinucleate meronts (up to 8 nuclei). Chains of transitional stages	Wang *et al*. 1991
N. pyrgomorphae	*Pyrgomorpha conica, P. cognata, P. bispinosa* (Pyrgomorphidae)	Mg, A, M, S, G	3.9 x 2 3.4 x 1.2	7-9	Multinucleate transitional stages (up to 8 nuclei), frequently moniliform	Toguebaye *et al.* 1988, Lange *et al.* 1992
N. trilophidiae	*Trilophidia annulata* (Acrididae: Oedipodinae)	S, Mg, G, B, N, T, A	3.7 x 1.6	8	No information	Wen 1996

other *Nosema* species of Acrididae (Henry 1967, 1971; Wang *et al.* 1991; Lange *et al.* 1992).

Our study did not reveal any difference in the morphology of *N. locustae* while developing in the three different hosts employed. Since the hosts used are not only different species but also belong to distinct subfamilies (Melanoplinae, Oedipodinae, and Cyrtacanthacridinae), the results of the study strongly suggest that the morphology *N. locustae* remains constant regardless of the host involved. Host induced morphological plasticity has been reported in other microsporidia (Walters 1958, Armstrong *et al.* 1986, Mercer and Wigley 1987, Hayasaka and Kawarabata 1990), but appears not to be a trait of *N. locustae.* That the morphological characters of *N. locustae* remains the same regardless the host involved, at least for the isolates of North American origin, is a desirable characteristic from an applied point of view because the diagnosis after introductions will be much reliable.

Ten species of the genus *Nosema* Naegeli have been described from Orthoptera hosts (Table 1). The published information on the structure of *N. chorthippi* Issi and Krylova, *N. maroccanus* Issi and Krylova, and *N. trilophidia* Wen structure is not sufficient for a comparative analysis. *Nosema montanae* Wang *et al.* and *N. asiaticus* Wen form multinucleate merogonial plasmodia and thus can be easily differentiated from *N. locustae.* The ultrastructure of *N. locustae* was similar to the following species: *N. pyrgomorphae* Toguebaye *et al.* from grasshoppers of the genus *Pyrgomorpha* Serville, *N. cuneatum* Henry and *N. acridophagus* Henry, both studied ultrastructurally from grasshoppers of the genus *Melanoplus* Stål, and *N. grylli* Sokolova *et al.* from the cricket *Gryllus bimaculatus* Deg. (Issi end Krylova 1987; Street and Henry 1987, 1993; Toguebaye *et al*. 1988; Lange *et al*. 1992; Sokolova *et al*. 1994, 1998; Wen 1996). The meront fine structure is quite similar in all five species, though *N. acridophagus* meronts are elongated and possess fine tubular elements on the plasmalemma. The deposition of the homogenous electron dense material, deriving from the host cell cytoplasm, on the external part of the sporont plasmalemma is characteristic for *N. locustae*, *N. acridophagus* and *N. grylli*. The ultrastructure of sporonts and of the transitional (meront/sporont) cells with a specific state of the nucleus, containing electron dense particles, is very similar in *N. locustae* and *N. grylli* (Sokolova *et al.* 1998). At the same time, the spore of *N. locustae* can be easily distinguished from other spores of orthopteran "Nosemas" by the largest size in the group, and 17-18 polar filament coils. *Nosema grylli* is the only species that possess a similar number of coils (18-20) but it is parasitic for *G. bimaculatus*, which is not susceptible to *N. locustae* (Y. Sokolova, unpublished observation). Compared with *N. pyrgomorpha*, *N. locustae* differs in host and tissue specificity (Table 1).

The peculiar presumably transitional (meront/sporont) stage of microsporidian life cycle, characterized by the nucleus filled with numerous electron dense intranuclear bodies have been mentioned so far only for *N. grylli* (Sokolova *et al.*1998). The same condition of nucleoplasm can be distinguished also in *N. pyrgomorphae* meronts (Fig.14 in Lange *et al*.1992). We believe this phase of nuclear cycle indicates the switch of parasite metabolism from proliferative (merogony) to morphogenic (sporogony) phase with intensification of biosynthetic processes, demanding rapid RNA processing and transcription. We suppose that electron dense intranuclear particles represent ribonucleoproteins (RNP). Their identification as well as investigation of their role in microsporidian host cycle is currently in progress. Among protists "RNP granules" are described from macronuclei of Infusoria and from nuclei of Foraminifera (Raikov 1982).

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