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Ultrastructure and molecular characterization of the microsporidium, *Nosema chrysoperlae* sp. nov., from the green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) used for biological pest control

S. Bjørnson [a,*], T. Steele [a], Q. Hu [a], B. Ellis [a], T. Saito [b]

[a] Department of Biology, Saint Mary's University, 923 Robie Street, Halifax, NS B3H 3C3, Canada
[b] Vineland Research and Innovation Centre, 4890 Victoria Ave. N., Box 4000, Vineland Station, ON L0R 2E0, Canada

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ABSTRACT

Lacewing larvae are generalist predators that are commercially available for aphid control on a variety of crops in both Europe and North America. Although lacewings are known for their symbiotic association with yeasts and bacteria, there are few reports of microsporidia in these natural enemies. An undescribed microsporidium was found in *Chrysoperla carnea* (Stephens) during the routine examination of specimens that were obtained from a commercial insectary for biological pest control. The objective of this study was to describe the pathogen by means of ultrastructure, molecular characterization and tissue pathology. All stages of the microsporidium were diplokaryotic and developed in direct contact with the host cell cytoplasm. Merogony and sporogony were not observed. Mature spores measured $3.49 \pm 0.10 \times 1.52 \pm 0.05$ μm and had an isofilar polar filament with 8–10 coils that were frequently arranged in a single row, although double rows were also observed. Spores contained a lamellar polaroplast and a relatively small and inconspicuous polar vacuole was observed in the posterior region of about half of the spores that were examined. Tubular structures, similar in appearance to those in *Nosema granulosis* were observed in both sporonts and in spores. A cluster of small tubules was also observed in the posterior region of some spores. Microsporidian spores were observed in cells of the proventriculus, diverticulum and in epithelial cells of the posterior midgut. The Malpighian tubules, ileum, and rectum were heavily infected. Spores were also observed in the fat body, peripheral region of the ganglia, within and between the flight muscles, and beneath the cuticle. Although the tissues adjacent to the ovaries were heavily infected, microsporidian spores were not observed within the developing eggs. Pathogen transmission was not studied directly because it was difficult to maintain microsporidia-infected *C. carnea* in the laboratory. The presence of microsporidian spores in the alimentary canal suggests that the pathogen is transmitted *per os* and horizontal transmission may occur when infected larvae or adults are cannibalized by uninfected larvae. Molecular analysis of the microsporidian genome showed that the pathogen described in this study was 99% similar to *Nosema bombycis*, *N. furnacalis*, *N. granulosis* and *N. spodopterae*. Based on information gained during this study, we propose that the microsporidium in *C. carnea* be given the name *Nosema chrysoperlae* sp. nov.

1. Introduction

During his trip to Australia in 1888, Albert Koebele was first to observe the predaceous behavior of lacewing larvae as they fed on the cottony cushion scale, *Icerya purchasi* Maskell (Koebele, 1890). The biological control potential of lacewings was soon fully appreciated and mass-culturing techniques for inundative release were developed (Finney, 1950). Today, both *Chrysoperla carnea* (Stephens) and *C. rufilabris* (Burmeister) are mass-produced for aphid control on various crops in Europe and North America (van Lenteren et al., 1997).

Lacewings are well known for their symbiotic association with yeasts, bacteria and filamentous fungi. Yeasts of the genera *Candida* and *Metschnikowia* are commonly reported in lacewings (Suh et al., 2004; Woolfolk and Inglis, 2004; Nguyen et al., 2006) and although the specific role of yeasts is poorly understood, they are thought to provide the host with a source of nutrients. More than 25 taxa of bacteria have been isolated from the alimentary canal of adult *C. rufilabris* and both larval and adult *C. carnea*. Dense populations

* Corresponding author. Fax: +1 902 420 5261.
E-mail address: susan.bjornson@smu.ca (S. Bjørnson).

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of these microbes in the midgut of *C. carnea* larvae suggests that bacteria are involved in the decomposition of food or are otherwise beneficial for larval growth and development (Chen et al., 2006). Filamentous fungi from six taxa have also been isolated from *C. rufilabris*. The infrequent presence of bacteria and filamentous fungi in the alimentary canal of chrysopids suggests that these microbes are transient and are unlikely to form symbiotic relationships with lacewings (Woolfolk and Inglis, 2004).

There is only one report of an infection caused by microsporidia in lacewings. In 1949, the microsporidium *Plistophora californica* Steinhaus & Hughes, a pathogen of the potato tuberworm, *Gnorimoschema operculella* (Zeller) was noted to infect several other insect hosts, including *Chrysoperla californica* Coquillet (Steinhaus and Hughes, 1949). At that time, the potato tuberworm was a significant pest when rearing mealybugs as food for the mass-production of the predatory coccinellid, *Cryptolaemus montrouzieri* Mulsant (see Finney et al., 1947). Although *C. californica* is a non-target host of *P. californica*, infection reduces lacewing longevity and fecundity (Finney, 1950).

In this study, we describe a microsporidium from *Chrysoperla carnea* that was detected during the examination of specimens that were obtained from a commercial insectary for biological pest control. This is the first report and formal description of a microsporidium from *C. carnea*. The objective of this study was to describe the pathogen by means of its ultrastructure, molecular characterization and tissue pathology.

2. Materials and methods

In 2010, *C. carnea* larvae were purchased from a European commercial insectary for use in a laboratory study. During the rearing process, some larvae turned black and died in their second- or third-instar. A microsporidian pathogen was detected in smear preparations of these individuals when examined by light microscopy.

In an attempt to study the transmission and effects of the pathogen, additional *C. carnea* larvae were reared in 47 mm-diameter Petri dishes (Millipore) under controlled conditions (16:8 L:D; 25 °C:20 °C). Each dish had a 2.2 cm hole cut in the lid that was covered with a fine mesh screen (80 μm), allowing air circulation. One larva was reared per dish. Larvae were fed green peach aphids (*Myzus persicae* Sulzer) and distilled water was provided through a moistened cotton wick (Crosstex International, NY). Individuals from aphid colonies were screened for microsporidia when the laboratory colony was established and on a routine basis during the experimental trials. Larvae were reared to adult and upon eclosion, some adults exhibited malformed wings that had a characteristic 'clubbed' appearance. Both asymptomatic and symptomatic adult specimens were processed for histological and microscopic examination. Specimens were also sent for sequencing to determine the molecular characterization of the pathogen. Attempts to rear microsporidia-infected *C. carnea* for further study were unsuccessful and vertical transmission was not confirmed.

2.1. Pathogen ultrastructure

Adult specimens ($n = 23$) reared individually from symptomatic mothers (those with 'clubbed' wings) were embedded for examination by transmission electron microscopy (TEM). Only two of these individuals were confirmed to be infected with the microsporidium and both were examined by TEM. Adult *C. carnea* were submerged and dissected in 2.5% glutaraldehyde. The head, wings and legs were removed from each specimen to encourage infiltration of the fixative. The thorax of each specimen was removed from its abdomen and each was processed separately. Tissues were stored in fixative (4 °C; 48–72 h) until they were processed according to the procedure by Becnel (1997) with the following modification: tissues were placed under vacuum (15 psi) overnight once they were in pure resin. Tissues were embedded in Jembed 812/Spurr resin (1:1, Canemco Inc., QC). Specimens were processed in two batches.

Ultra-thin (70 nm) sections cut with a Leica UCT ultramicrotome were stained with uranyl acetate and lead citrate prior to examination. Digital micrographs were generated with a GATAN ES500W Erlangshen CCD camera side mounted to a Hitachi H7500 transmission electron microscope at 80 KV. Imaging software (ImageJ) was used for determining spore measurements.

2.2. Tissue pathology

Fifteen adult *C. carnea* were embedded for histological examination and a total of 10 were examined (4 uninfected and 6 microsporidia-infected). Each specimen was submerged and dissected in Carnoy's fixative (60 ml ethanol, 30 ml chloroform, 10 ml glacial acetic acid). The head, wings and legs were removed from each individual but the thorax was left attached to the abdomen. Prior to processing, a small piece of tissue excised from the anterior region of the prothorax was examined to confirm infection by light microscopy.

Specimens were stored in fixative (4 °C; 48–72 h) until they were processed according to the procedure by Becnel (1997), with the following modifications: (1) tissues remained submerged in 1:1 ethanol:butanol overnight in a >25.5 °C oven (instead of 2 h at room temp); (2) tissues were submerged in 3:1 butanol:paraffin for 20 min in a 60 °C oven (this step was inserted between 100% butanol and 1:1 butanol:paraffin); and (3) tissues remained in 1:1 butanol:paraffin overnight (instead of 2 h). Tissues were embedded in Paraplast® Plus (Sigma–Aldrich Inc., melting point 56 °C). Specimens (both uninfected and infected) were processed simultaneously in three batches.

Serial, longitudinal sections (5 μm thickness) cut with a rotary microtome were fixed to slides that had been pre-coated with a protein solution (1 g gelatin, 2 g solid phenol, 15 ml glycerin, 100 ml distilled water) to enhance fixation. Once dry, the paraffin was removed with xylene and sections were rehydrated with solutions of ethanol in decreasing concentration until distilled water. Tissues were stained with Harris hematoxylin and Alcoholic Eosin Y (Fisher Scientific) and examined by light microscopy.

2.3. Molecular characterization

Microsporidia genomic DNA was extracted from *C. carnea* adults according to the methods described by Bjørnson et al. (2011). DNA was isolated and purified with a DNeasy® Blood & Tissue Kit (Qiagen) following the manufacturer's protocol. Microsporidian primers described by Vossbrinck et al. (2004) were used to design primers for this study (18f (CACCAGGTTGATTCTGCC)/1492 (GGTTACCTTGTTACGACTT); Eurofins Laboratories). DNA was amplified by Polymerase Chain Reaction (PCR), cloned using pGEM®-T and pGEM®-T Easy Vector Systems (Promega Corp.), extracted with PureYield™ Plasmid Miniprep System (Promega Corp.), and sent to Macrogen (Korea) for sequencing.

Sequences of *Nosema bombycis*, *N. furnacalis*, *N. granulosis* and *N. spodopterae* were obtained from NCBI GeneBank. *N. apis* was used for comparison as an outgroup. Automated sequence outputs were visually inspected with chromatographs. Multiple sequence alignments were made using ClustalX with default parameters and additional manual edits to minimize gaps (Thompson et al., 1997). Phylogenetic analysis using the maximum-parsimony (MP) method was performed with the computer program PAUP* ver. 4 beta 10 (Swofford, 2003). All characters were specified as un-

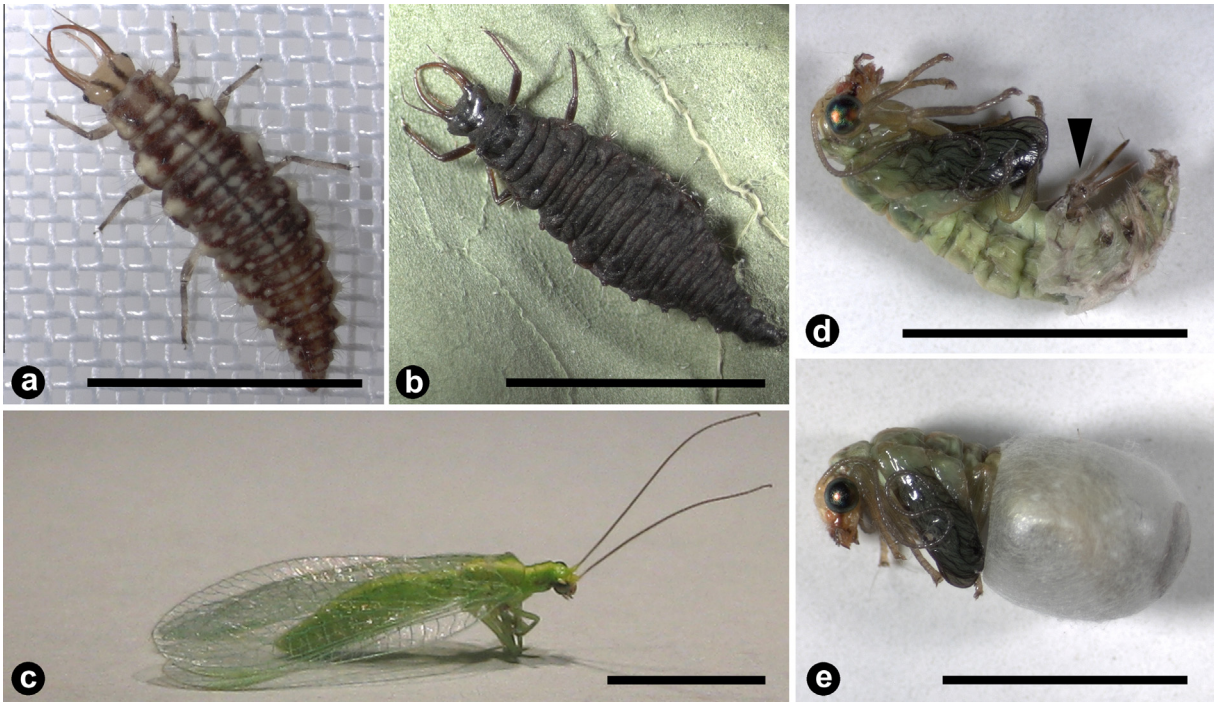


Fig. 1. *Chrysoperla carnea* third-instar larvae and adults: (a) Live, uninfected larva; (b) Dead, microsporidia-infected larva that turned black as a result of infection; (c) Live, uninfected *C. carnea* adult; (d) Dead, partially eclosed, microsporidia-infected adult with 'clubbed' wings. The pupal case is lacking but the larval exuvia, including the larval head capsule (arrow) remains attached; (e) Dead adult with deformed, 'clubbed' wings that was unable to eclose from its pupal case. Scale bars: 0.5 mm.

weighted and unordered, and gap-only columns were excluded in the analyses. The most parsimonious trees were constructed by performing a heuristic search using Tree Bisection-Reconnection (TBR) with the following parameters: MulTrees on and 10 replications of random addition sequences with the stepwise addition option. Multiple parsimonious trees were combined to form a strict consensus tree. Overall character congruence was estimated by the consistency index (CI) and the retention index (RI). To infer the robustness of clades, bootstrap values with 1000 replications (Felsenstein, 1985) were calculated by performing a heuristic search using the TBR option with MulTree on.

In addition to MP analysis, maximum-likelihood (ML) and Bayesian analyses were also performed. For the ML analysis, eight nested models of sequence evolution were tested for each data set using PhyML 3.0 (Guindon and Gascuel, 2003). For each data set, the general time-reversible (GTR) (Lanave et al., 1984) substitution model led to a largest ML score compared to the other seven substitution models: JC69 (Jukes and Cantor, 1969), K80 (Kimura, 1980), F81 (Felsenstein, 1981), F84 (Felsenstein, 1993), HKY85 (Hasegawa et al., 1985), TN93 (Tamura and Nei, 1993) and custom (data not shown). As a result, the GTR model was used in the Bayesian analysis using MrBayes 3.1 (Ronquist and Huelsenbeck, 2005). MrBayes 3.1 was run with the program's standard setting of two analyses in parallel, each with four chains, and an estimated convergence of results was determined by calculating the standard deviation of split frequencies between analyses. In order to make the standard deviation of split frequencies fall below 0.01, so that the occurrence of convergence could be certain, 110,000 generations data were run for small subunit rRNA gene. Samples were taken every 1000 generations under the GTR model with gamma-distributed rate variation across sites and a proportion of invariable sites. For all analyses, the first 25% of samples from each run were discarded as burn-into ensure the stationarity of the chains. Bayesian posterior probability (PP) values were obtained from a majority rule consensus tree generated from the remaining sampled trees.

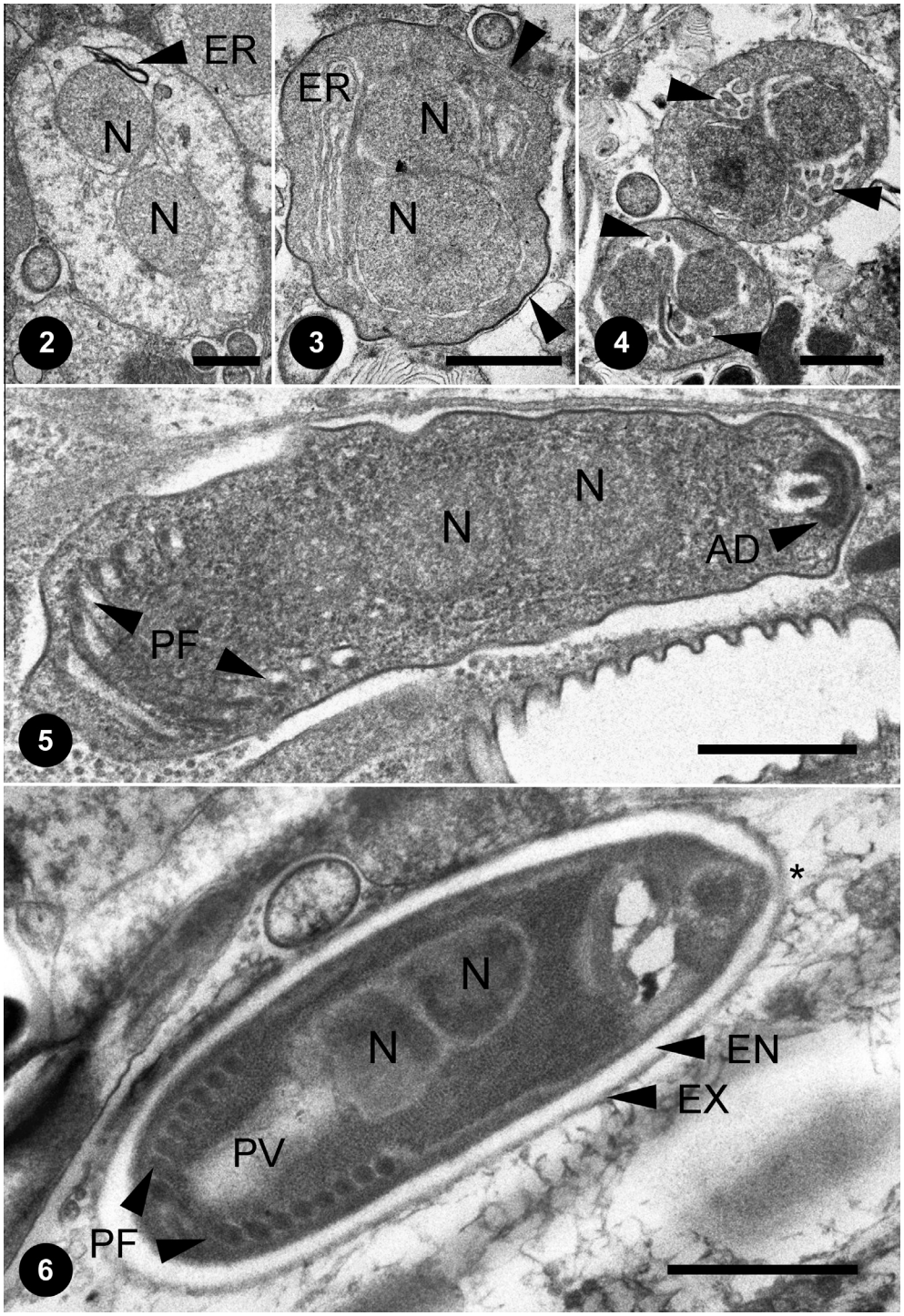
3. Results

Microsporidia were not detected in the aphids that were examined from the colonies used as food for *C. carnea*. Some *C. carnea* larvae that were reared in the lab turned from mottled ivory and reddish-brown[1] (Fig. 1a) to black (Fig. 1b). These specimens died prematurely, often late in their second- or third-instar. Smear preparations of these larvae revealed microsporidian spores. Some of the microsporidia-infected larvae did not turn black and these individuals developed successfully to eclose as adults. Uninfected green lacewing adults have characteristic, long and delicate wings with network venation (Fig. 1c). In contrast, microsporidia-infected adults were often identified by their distinctive, malformed wings that had a characteristic 'clubbed' appearance (Fig. 1d and e). In such cases, the wings appeared to be fused into a mass of solid tissue. Some of these infected adults were unable to eclose successfully and died with remnants of larval exuvia attached to their bodies or were otherwise unable to emerge completely from their pupal case.

3.1. Pathogen ultrastructure

All stages of the microsporidium developed in direct contact with the host cell cytoplasm. Round to oval meronts (Fig. 2) contained a cytoplasm with free ribosomes that was surrounded by a thin, plasma membrane. Diplokaryotic nuclei were primarily observed (80/152 observations; 52.6%). However, meronts with one, three or four nuclei were observed (66/152 observations, 43.4%; 4/152, 2.6%; and 2/152, 1.3%, respectively) but it is likely that these observations were sectioning artifacts. The nuclei of meronts occupied two thirds of the cell and there was evidence of an early-developing endoplasmic reticulum around the nuclear perimeter. Merogony was not observed.

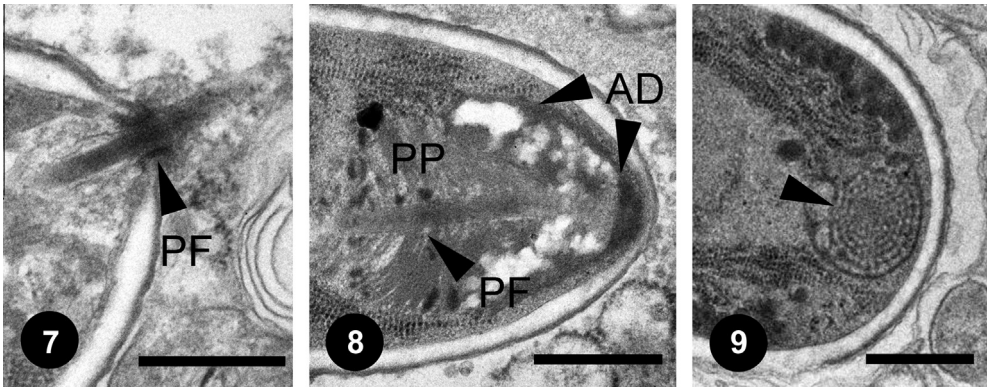
[1] For interpretation of color in Fig. 1, the reader is referred to the web version of this article.



Figs. 2–6. Vegetative stages and mature spores of *Nosema chrysoperlae* sp. nov. Fig. 2. Diplokaryotic meront in direct contact with the host cell cytoplasm. The meront cytoplasm contains free ribosomes, a developing endoplasmic reticulum (ER), two nuclei (N) and is surrounded by a thin plasma membrane. Fig. 3. Sporont with a developing thick outer membrane (arrows), well-developed endoplasmic reticulum (ER) and diplokaryon (N). Fig. 4. Diplokaryotic sporonts showing tubular structures (arrows) within the cytoplasm. Fig. 5. Sporoblast with a diplokaryon (N), showing development of the anchoring disk (AD) and polar filament (PF). Fig. 6. Mature spore with a fully-developed cell wall, composed of an exospore (EX) and endospore (EN), a well-defined diplokaryotic nucleus (N), and an isofilar polar filament (PF) arranged in a single layer. A rather inconspicuous polar vacuole (PV) is evident within the posterior end of the spore. Thinning of the spore wall is visible at the spore apex (∗). Scale bars: 1 μm.

Sporonts (Fig. 3) were oval to irregular in shape. The diplokaryon occupied a large, central region of the cell and was surrounded by a well-developed endoplasmic reticulum. Condensed chromatin was often visible within the nuclei. The thickened plasma membrane was often undulated in appearance and the cytoplasm contained more free ribosomes than did the meront. Tubular structures were observed in sporonts (24/61 observations, 39.3%; Fig. 4) as well as in spores (7/37, 18.9%).

Sporoblasts (Fig. 5) were highly irregular in shape. They were surrounded by a thickened, somewhat convoluted plasma membrane and contained a diplokaryon. The cytoplasm was filled with free ribosomes and contained endoplasmic reticulum and a devel-



Figs. 7–9. Ultrastructural detail of the anterior and posterior regions of mature spores of *Nosema chrysoperlae* sp. nov. Fig. 7. Ejection of the polar filament (PF) through the apex of a mature spore. Fig. 8. The anchoring disk (AD), lamellar polaroplast (PP) and polar filament (PF) located within the apical region of a mature spore. Fig. 9. A cluster of small tubules (arrow) in the posterior region of a mature spore. Scale bars: 0.5 μm.

oping polar filament and anchoring disk. Sporogony was not observed.

Diplokaryotic spores (Fig. 6) measured 3.49 ± 0.10 × 1.52 ± 0.05 μm (±SE; n = 37, from micrographs). The isofilar polar filament was arranged in 8–10 coils (n = 34) with three exceptions (when 5, 6, or 11 coils were observed). The polar filament was frequently arranged in a single row (29/37 observations; 78.4%) but double rows were also observed (8/37; 21.6%). There was evidence of polar filament extrusion within the host tissues (Fig. 7) and evacuated (germinated) spores were observed (data not shown). Mature spores had a lamellar polaroplast (Fig. 8) and an anchoring disk with relatively long arms. A relatively small and inconspicuous polar vacuole was observed in about half of the spores that were examined (19/37 observations). Spores were surrounded by a well-developed spore wall and a cluster of small tubules was often observed in the posterior region of the spore (18/37 observations, 48.6%; Fig. 9).

3.2. Tissue pathology

Four of the six microsporidia-infected specimens examined were female. The microsporidium infected several tissues of both male and female specimens. Microsporidian spores were observed in cells of the proventriculus and diverticulum (foregut, not shown). When intact, the epithelial cells in the anterior region of the midgut were uninfected whereas those in the posterior region contained few microsporidian spores. In four of six cases, the midgut epithelial cells were damaged severely as a result of infection. Damaged cells did not have any microvilli and often only small remnants of these cells remained. The Malpighian tubules, ileum, and rectum (hindgut; Fig. 10) were heavily infected and large numbers of microsporidian spores filled these cells.

The fat body and peripheral region of the thoracic and abdominal ganglia (Fig. 11) were also infected and spores occupied regions within and between the flight muscles (Fig. 12). Spores were observed beneath the cuticle and in one case they were observed in the tissue surrounding the trachea (Fig. 13). Although the tissues adjacent to the ovaries were heavily infected, microsporidian spores were not observed within the developing eggs in any of the females examined. Spores were observed in the gonads of one of the two males examined.

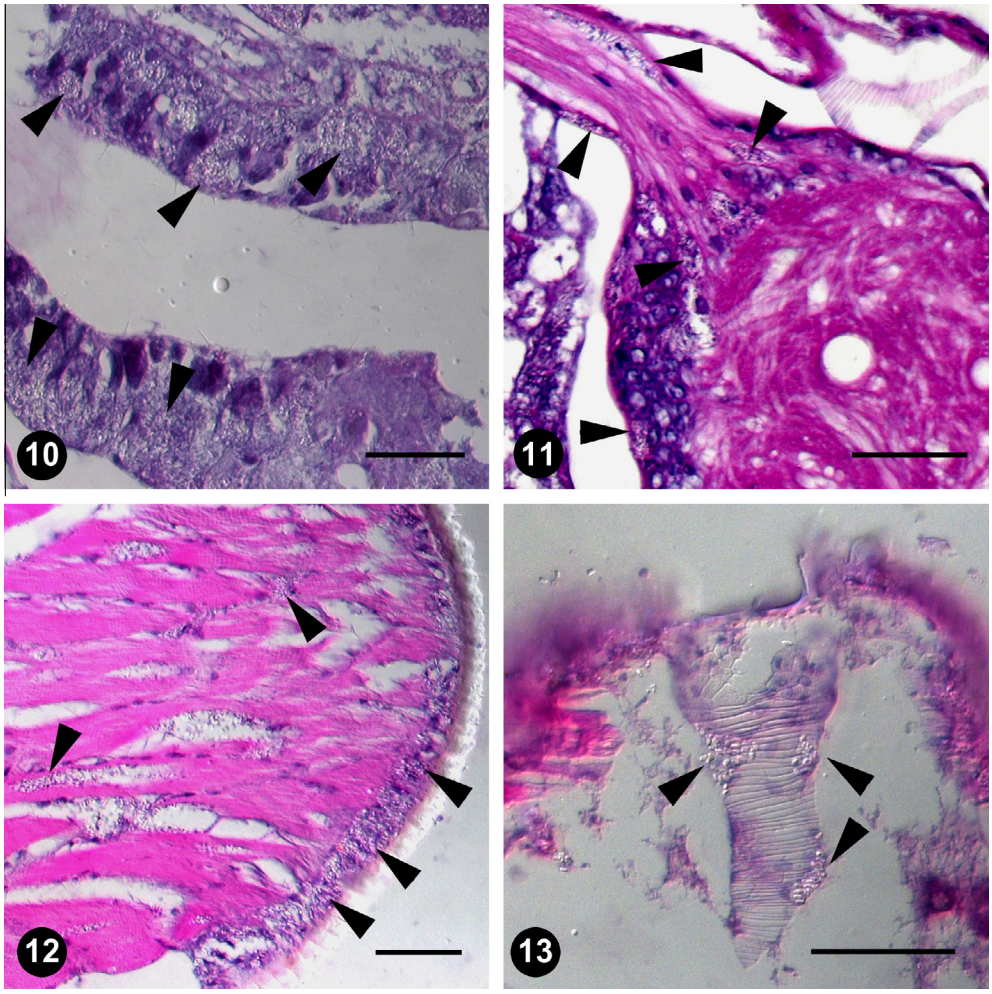
3.3. Molecular characterization

Molecular analysis of the genome showed the pathogen described in this study was 99% similar to *N. bombycis* (Accession No.: AY209011.1), *N. furnacalis* (Accession No.: U26532.1), *N. granulosis* (Accession No.: AJ011833.1) and *N. spodopterae* (Accession No.: AY747307.1). Maximum parsimony analysis using *N. apis* as the outgroup was conducted (26 parsimony-informative characters, 483 equally most parsimonious trees (CI = 0.981, RI = 0.743). The separated Bayesian analyses using GTR model resulted in identical trees with mean log-likelihood values −3008.69 and −3013.82 (data not shown). The tree topologies were identical in both ML and Bayesian trees and similar to those generated by MP. Strict consensus trees with bootstrap (1000 replicates) value and Bayesian PP showed two different clades (Fig. 14). The microsporidium described in this study formed one clade with *N. furnacalis* and *N. granulosis* with 74% bootstrap support. Within this clade, *N. furnacalis* and *N. granulosis* formed a subclade. *N. bombycis* and *N. spodopterae* formed a separate clade (BS = 98%, PP = 0.96).

4. Discussion

Microsporidia are common pathogens of natural enemies, including those that are mass-produced and commercially available for biological pest control (Bjørnson and Schütte, 2003). Microsporidia have been reported in the predatory mites *Phytoseiulus persimilis* Athias-Henriot (Bjørnson et al., 1996; Bjørnson and Keddie, 2000), *Neoseiulus cucumeris* Oudemans and *N. barkeri* Hughes (Beerling and van der Geest, 1991), *Metaseiulus occidentalis* (Nesbitt) (Becnel et al., 2002) as well as the pteromalid endoparasitoid *Muscidifurax raptor* Girault and Saunders (Geden et al., 1992). Microsporidia are also pathogens of field-collected convergent lady beetles, *Hippodamia convergens* Guérin-Méneville (Bjørnson et al., 2011) that are redistributed for aphid control. The microsporidium described from *C. carnea* is yet another example of a cryptic pathogen from a mass-produced natural enemy that may have otherwise gone unnoticed if not for the routine examination of individuals for pathogens.

Three of the four microsporidia that are most closely related to the microsporidium found in *C. carnea* infect lepidopteran hosts. *N. bombycis* is perhaps best known for causing pébrine disease in silkworms, *Bombyx mori* L. (Tanada and Kaya, 1993) but this pathogen also infects other lepidopterans, most notably the small and large white, *Pieris rapae* L. and *P. brassicae* L., respectively (Kashkarova and Khakhanov, 1980). *N. furnacalis* was initially described from the Asian corn borer, *Ostrinia furnacalis* (Guenée) (see Oien and Ragsdale, 1993) and *N. spodopterae* is a pathogen of the tobacco cutworm *Spodoptera litura* Fabr. (see Johny et al., 2006). In contrast, *N. granulosis* is the only pathogen that is closely related to the microsporidium in *C. carnea* to infect an amphipod, *Gammarus duebeni* Liljeborg (see Terry et al., 1999).



Figs. 10–13. *Chrysoperla carnea* tissues infected with *Nosema chrysoperlae* sp. nov. Fig. 10. Microsporidian spores (arrows) within cells in the rectum. Fig. 11. Microsporidian spores (arrows) in the peripheral region of the thoracic ganglion. Fig. 12. Microsporidian spores within the flight muscles (arrows, left) and beneath the cuticle (arrows, right). Fig. 13. Microsporidian spores (arrows) in the in the tissue surrounding the trachea. Scale bars: 20 μm.

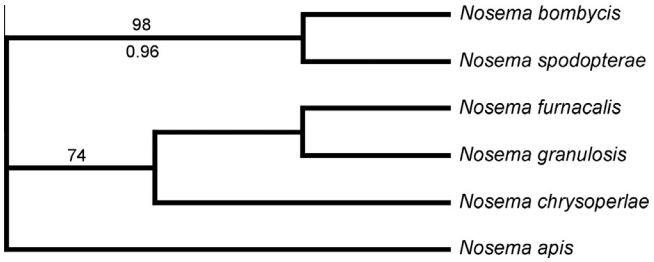


Fig. 14. Strict consensus tree derived from 16S ribosomal RNA gene sequence data conducted using a heuristic search with TBR branch swapping. Numbers above and below branches are bootstrap values and Bayesian posterior probability (PP) values, respectively. *Nosema apis* was used as an out group. Consistency index (CI) = 0.981, retention index (RI) = 0.743.

Some infected *C. carnea* larvae and adults showed distinct signs associated with infection. Some larvae turned black during the latter part of their development and died but others were able to complete development and eclose successfully as adults. Many of these infected adults had deformed wings that had a distinct 'clubbed' appearance. In contrast, the microsporidium *Plistophora californica* infects both the larvae of the potato tuber worm (*G. operculella*) and *C. californica*. Heavily infected *G. operculella* larvae are somewhat opaque and whitish in appearance; however, there is no mention of any signs associated with infection of *C. californica* (Steinhaus and Hughes, 1949).

4.1. Pathogen ultrastructure

Spores of the microsporidium in *C. carnea* measured 3.49 ± 0.10 × 1.52 ± 0.05 μm and were larger than those of *P. californica* (1.5–3.0 × 0.8–1.2 μm; mean dimensions: 2.0×1.0 μm; Steinhaus and Hughes, 1949). Spores in *C. carnea* had a relatively small polar vacuole, a characteristic of *Nosema* sp. (Sprague et al., 1992) and all stages of the pathogen were diplokaryotic and developed in direct contact with the host cell cytoplasm. Vegetative stages and spores contained tubular structures; those in the vegetative stages were similar in appearance to those in *Nosema granulosis* (see Terry et al., 1999).

In the case of *P. californica*, sporonts have one to four nuclei and in most cases, they develop into multi-nucleated, plasmodia-like structures (pansporoblasts) that contain several nuclei. Each nucleus develops into a mature spore. Steinhaus and Hughes (1949) provide diagrams of *P. californica* development and although the number of nuclei per spore is not stated, the diagrams depict one nucleus per spore. Individual *P. californica* spores or groups of spores (most with 16 spores; range: 8 to >100 spores per group) are observed in fresh specimens. Although merogony and sporogony were not observed in the microsporidium from *C. carnea*, there

was no evidence of plasmodia-like structures similar to what was described for *P. californica*.

4.2. Tissue pathology

Microsporidian spores were observed in the foregut (proventriculus and diverticulum), the posterior region of the midgut, and the hindgut (Malpighian tubules, ileum, and rectum). As a result of infection, the midgut epithelial cells often lacked microvilli and few cells remained intact. The presence of spores in the alimentary canal, particularly in the Malpighian tubules and rectum, supports the assumption that this pathogen is transmitted *per os*. Horizontal transmission may occur though several mechanisms but because cannibalism is common among lacewing larvae, the pathogen is likely transmitted when infected larvae or infected adults are eaten by uninfected larvae. Although the pathogen is closely related to *N. furnacalis*, the latter does not infect *C. carnea* larvae when they are fed spore solutions under laboratory conditions (Oien and Ragsdale, 1993). Polar filament extrusion (Fig. 7) and evacuated (germinated) spores within *C. carnea* tissues provide evidence that autoinfection does occur.

Based on the information on pathogen ultrastructure and the molecular information gained during this study, we propose that this previously undescribed species of microsporidia in *C. carnea* be considered a new species and be given the name *Nosema chrysoperlae* sp. nov.

4.2.1. Taxonomic Summary (Nosema chrysoperlae)

Nosema chrysoperlae sp. nov., Bjørnson, Steele, Hu, Ellis & Saito GenBank Accession Number KC412707.

Type host: *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae).

Other hosts: unknown.

Type locality: *C. carnea* purchased from a European commercial insectary for biological pest control.

Site of infection: Malpighian tubules, ileum, and rectum (hindgut) are heavily infected. Other infected tissues include the proventriculus, diverticulum, epithelial cells (posterior midgut), fat body, ganglia, and flight muscles. Spores were observed in the gonads of one of two males examined but not within developing eggs.

Transmission: autoinfection was observed. Infection of the alimentary canal suggests that horizontal transmission occurs *per os*. There is no evidence to confirm vertical transmission.

Merogony: not observed.

Sporogony: not observed.

Interface: all stages of the microsporidium developed in direct contact with the host cell cytoplasm.

Spores: diplokaryotic, 3.49 ± 0.10 x 1.52 ± 0.05 μm (n = 37, from micrographs) with a lamellar polaroplast, relatively inconspicuous polar vacuole and a polar filament arranged in 8–10 coils (n = 34) in a single (or occasionally double) row. Clusters of tubular structures were observed in both vegetative stages and spores.

Etymology: specific name refers to the host genus *Chrysoperla*, from which the pathogen was described.

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