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Nosema pieriae sp. n. (Microsporida, Nosematidae): A New Microsporidian Pathogen of the Cabbage Butterfly *Pieris brassicae* L. (Lepidoptera: Pieridae)

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Abstract. A new microsporidian pathogen of the cabbage butterfly, *Pieris brassicae* is described based on light microscopy, ultrastructural characteristics and comparative small subunit rDNA analysis. The pathogen infects the gut of *P. brassicae*. All development stages are in direct contact with the host cell cytoplasm. Meronts are spherical or ovoid. Spherical meronts measure 3.68 ± 0.73 × 3.32 ± 1.09 µm and ovoid meronts 4.04 ± 0.74 × 2.63 ± 0.49 µm. Sporonts are spherical to elongate (4.52 ± 0.48 × 2.16 ± 0.27 µm). Sporoblasts are elongated and measure 4.67 ± 0.60 × 2.30 ± 0.30 µm in length. Fresh spores with nuclei arranged in a diplokaryon are oval and measure 5.29 ± 0.55 µm in length and 2.31 ± 0.29 µm in width. Spores stained with Giemsa's stain measure 4.21 ± 0.50 µm in length and 1.91 ± 0.24 µm in width. Spores have an isofilar polar filament with six coils. All morphological, ultrastructural and molecular features indicate that the described microsporidium belongs to the genus *Nosema* and confirm that it has different taxonomic characters than other microsporidia infecting *Pieris* spp.

Key words: *Pieris brassicae*, microsporidian pathogen, *Nosema*, Lepidoptera, biological control.

INTRODUCTION

The large white butterfly, *Pieris brassicae* (Linnaeus) (Lepidoptera: Pieridae) is the most serious pest of vegetables and it has spread to many countries. Its larvae feed on cabbage and other plants, and are a major constraint to vegetable production. It damages all the growing parts of the plant such as leaves, branches and pods (Rizvi *et al.* 2009). Fortunately, most of the micro-organisms capable of causing disease in insects do not have a deleterious effect on other animals or plants. Despite high numbers and a wide distribution of *P. brassicae*, very little is known about the microsporidian pathogens limiting its populations (Malone and McIvor 1996). In this study, the microsporidian pathogens of *P. brassicae* were investigated to obtain data for biological control. Here, we describe a new microsporidian pathogen of *P. brassicae*.

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MATERIALS AND METHODS

Insect samples

P. brassicae larvae were collected from June to August 2011 in Beşikdüzü (Trabzon), Turkey. The larvae were put into plastic boxes, brought to the laboratory and dissected as soon as possible.

Light microscopy

The larvae were dissected in Ringer's solution and wet smears were examined under a light microscope at magnifications of 400 × to 1000 × for detection of microsporidian pathogens (Yaman and Radek 2003). When an infection with a pathogen was observed, the slides were air-dried and fixed in methanol for 10 min. The slides were then washed with distilled water and stained for approximately 10 hours in freshly prepared 5% solution of Giemsa stain (stock solution, Carloerba, No. 6B712176C). Afterwards the slides were washed in running tap water, air-dried and re-examined under the light microscope (Undeen and Vavra 1997, Yaman *et al*. 2008). Detected spores were measured and photographed using an Olympus BX51 microscope with a DP-25 digital camera and a DP2-BSW Soft Imaging System.

Transmission electron microscopy

For transmission electron microscope studies, portions of infected larvae were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1–2 h, rinsed in cacodylate buffer, postfixed in reduced OsO_4 according to Karnovsky (1971) (a fresh 1:1 mixture of 2% OsO_4 and 3% $K_4[Fe(CN_6)]$) for 1.5 h, rinsed in cacodylate buffer, and dehydrated in ethanol prior to embeding in Spurr's resin (Spurr 1969). Thin sections were mounted on Pioloform-coated copper grids which were then stained with saturated uranyl acetate and Reynolds' lead citrate (Reynold 1963). They were examined with a Philips 208 transmission electron microscope (TEM).

Spore purification and genomic DNA extraction

Infected larvae were homogenized in Ringer's solution with a micropestle. The homogenates were filtered through three layers of cheesecloth and centrifuged at 295 g for 2 min (Chen *et al.* 2012). The spore pellet was diluted with distilled water and the spore number counted with hemocytometer (7.4×10^7 spore/ml).

An equal volume of spore suspension and H_2O_2 (0.3%) were added in a 1.5 ml Eppendorf tube and kept at room temperature for 15 min (Higes *et al.* 2006). After this process glass beads (0.425–0.600 µm) were added in the same tube and tube was vigorously shaken for 2–3 min at maximum speed on the vortex (Hylis *et al.* 2005). ATL buffer from QIAGEN DNA Isolation Kit (No. 69504) was added to an equal amount to the total volume and shaken with the vortex. Then 20 µl Proteinase K (No. P6556) was added and slightly shaken again with the vortex. The resulting suspension was incubated at 56°C for 3 hours. After the incubation, DNA extraction was performed with the QIAGEN DNA Isolation Kit, No. 69504 according to the manufacturer's guidelines.

Amplification and sequencing of SSU rRNA genes

To amplify the microsporidial SSU rRNA genes, the 18F/1537R primer set (18F/1537R: 5'-CACCA GGTTG ATTCT GCC-3'/5'-TTATG ATCCT GCTAA TGGTT C-3') and the QIAGEN Multiplex PCR Kit (No. 206143) were used (Vossbrinck *et al.* 1987, 1993). The amplification was performed under following condition: after initial denaturation of DNA at 95°C for 15 min, 45 cycles were run – 94°C for 30 s, 61°C for 90 s and 72°C for 90 s – with a 10 min at 72°C extension.

The PCR amplified products were loaded on 0.9% agarose gel which was supplemented with ethidium bromide (EtBr). Then the PCR products and the primers used for PCR were sent to the Macrogen Inc. Company, Netherland for determination of the base sequences.

Construction of phylogenetic tree and analysis of SSU rRNA gene sequence

SSU rRNA gene sequences from 37 microsporidians (Table 1) were aligned with an HKY85 substitution model of PAUP 4.0b10 software. One thousand bootstrap replicates and one hundred characters resampling were generated to test the stability of the tree. Subsequently the GC content of the SSU rRNA sequence of our microsporidium and other similar species sequences were analyzed with the FastPCR program.

RESULTS

A new microsporidian infection was found in the larvae of *Pieris brassicae* collected in the vicinity of Trabzon (Turkey). Two of the 30 dissected larvae were infected with that microsporidium. Light microscopic observations of the infected individuals revealed the presence of the pathogen only in the gut. During the examinations, we observed both spores and intracellular stages.

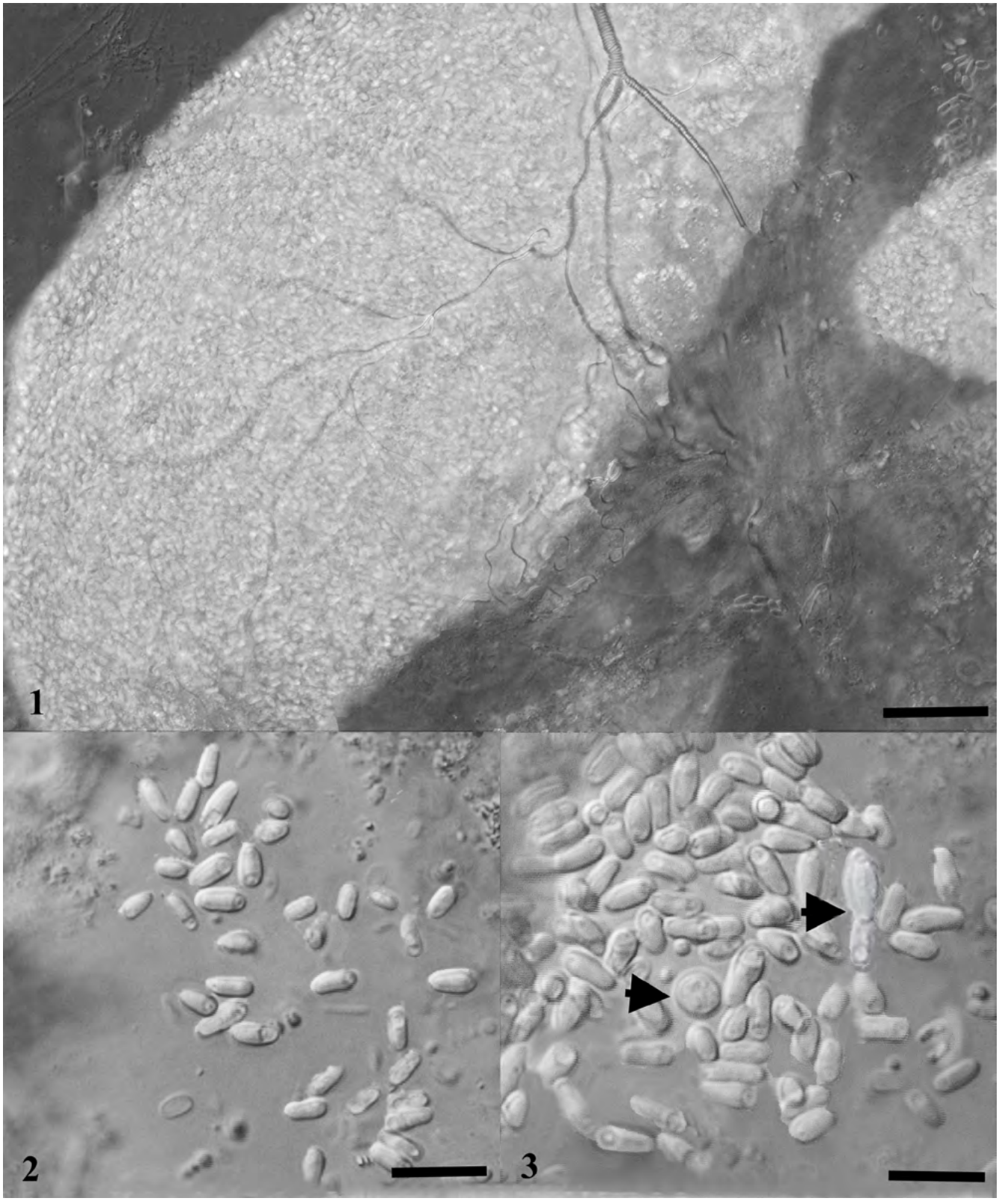
Developmental stages observed with light microscopy

Spores were observed in fresh smears of the intestine (Fig. 1). They were oval in shape and measured 5.29 ± 0.55 (3.71–7.26; n = 250) µm in length and 2.31 ± 0.29 (1.65–3.30; n = 250) µm in width (Figs 2, 3). Several life stages of the pathogen were observed in the Giemsa stained microscopic smears. Meronts were spherical or ovoid. The spherical meronts measured 3.68 ± 0.73 (2.89–4.78; n = 8) × 3.32 ± 1.09 (2.13–4.97; n = 8) µm, and ovoid meronts were 4.04 ± 0.74 (3.02–6.32; n = 30) × 2.63 ± 0.49 (1.66–4.09; n = 30) µm in size (Figs 4, 5). Sporonts were spherical to elongate and measured 4.52 ± 0.48 (3.46–5.62; n = 60) × 2.16 ± 0.27 (1.65–3.38; n = 60) µm. Diplokaryotic sporonts divided once to produce two sporoblasts which mature into spores. Sporoblasts were elongated and measured 4.67 ± 0.60 (3.48–5.98; n = 35) × 2.30 ± 0.30 (1.85–2.94; n = 35) µm (Fig. 6). Spores stained with Giemsa stain measured 4.21 ± 0.50 (3.13–5.47; n = 50) µm in length and 1.91 ± 0.24 (1.31–2.45; n = 50) µm in width.

Table 1. Small subunit (SSU) ribosomoal RNA sequences used for phylogenetic analysis.

Accession No	Organism name	Host	Order	Family
AF426104	*Nosema carpocapsae*	*Cydia pomonella*	Lepidoptera	Tortricidae
EU260046	*Endoreticulatus* sp. CHW-2008 Austria	*Thaumetopoea processionea*	Lepidoptera	Thaumetopoeidae
L39109	*Endoreticulatus schubergi*	*Homo sapiens*	Primates	Hominidae
AY009115	*Endoreticulatus bombycis*	*Bombyx mori*	Lepidoptera	Bombycidae
AY502944	*Endoreticulatus* sp. CHW-2004 Taiwan	*Ocinara lida*	Lepidoptera	Bombycidae
AJ011833	*Nosema granulosis*	*Gammarus duebeni*	Amphipoda	Gammaridae
DQ073396	*Nosema antheraeae*	*Antheraea pernyi*	Lepidoptera	Saturniidae
FJ767862	*Nosema philosamiae*	*Philosamia cynthia*	Lepidoptera	Saturniidae
D85502	*Vairimorpha* sp. NIS-M12	*B. mori*	Lepidoptera	Bombycidae
Y00266	*Vairimorpha necatrix*	*Psuedaletia unipuncta*	Lepidoptera	Noctuidae
U27359	*Nosema oulemae*	*Oulema melanopus*	Coleoptera	Chrysomelidae
D85501	*Nosema* sp. NIS-M11	*B. mori*	Lepidoptera	Bombycidae
DQ486027	*Nosema ceranae*	*Apis cerana*	Hymenoptera	Apidae
AF033315	*Vairimorpha lymantriae*	*Lymantria dispar*	Lepidoptera	Lymantriidae
AF033316	*Nosema portugal*	*Lymantria dispar*	Lepidoptera	Lymantriidae
EU219086	*Nosema thomsoni*	*Choristoneura conflictana*	Lepidoptera	Tortricidae
AY008373	*Nosema bombi*	*Bombus terrestris*	Hymenoptera	Apidae
U97150	*Nosema apis*	*Apis mellifera*	Hymenoptera	Apidae
AJ252955	*Pleistophora ovariae*	*Notemigonus crysoleucas*	Cypriniformes	Cyprinidae
AJ252956	*Pleistophora typicalis*	*Myoxocephalus scorpius*	Scorpaeniformes	Cottidae
D85500	*Pleistophora* sp. Sd-Nu-IW8201	*B. mori*	Lepidoptera	Bombycidae
AJ131646	*Vairimorpha imperfecta*	*Plutella xylostella*	Lepidoptera	Plutellidae
AY960987	*Nosema plutellae*	*Plutella xylostella*	Lepidoptera	Plutellidae
AF240350	*Nosema* sp.	*B. mori*	Lepidoptera	Bombycidae
AF485270	uncultured *Nosema*	*Pieris rapae*	Lepidoptera	Pieridae
AF240354	*Nosema* sp.	*Pieris rapae*	Lepidoptera	Pieridae
D85503	*Nosema bombycis*	*B. mori*	Lepidoptera	Bombycidae
AY259631	*Nosema bombycis*	*Helicoverpa armigera*	Lepidoptera	Noctuidae
AY747307	*Nosema spodopterae*	*Spodoptera litura*	Lepidoptera	Noctuidae
AY383655	*Nosema* sp. C01	*Pieris rapae*	Lepidoptera	Pieridae
AJ131645	*Vairimorpha imperfecta*	*Plutella xylostella*	Lepidoptera	Plutellidae
AF240351	*Nosema* sp.	*B. mori*	Lepidoptera	Bombycidae
AF025685	*Amblyospora connecticus*	------	Diptera	Glossinidae
L39114	*Vairimorpha* sp.	*B. mori*	Lepidoptera	Bombycidae
U09282	*Nosema trichoplusia*	*Trichoplusia ni*	Lepidoptera	Noctuidae
AF141129	*Vairimorpha lymantriae*	*Lymantria dispar*	Lepidoptera	Lymantriidae
HQ399665	*Nosema* sp.	*Pieris rapae*	Lepidoptera	Pieridae
JX268035	***Nosema pieriae* sp. n.**	***Pieris brassicae***	**Lepidoptera**	**Pieridae**

The microsporidian pathogen presented in this study is indicated in bold.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Spore ultrastructure

Electron microscopic observations confirmed that the oval spores contain a diplokaryon with spherical nuclei measuring 675–750 nm in diameter (Figs 7, 8). The spore wall was thin (100 to 125 nm); the exospore thickness was 25 to 34 nm and the endospore thickness was 80 to 95 nm. The polar filament was isofilar and had six coils (Figs 8, 10). Mature coils measured 57–71 nm in diameter. The well-developed polaroplast had a lamellated structure with thin lamellae (anterior) and thick lamellae (posterior) under the anchoring disc (Fig. 9). All stages lay in direct contact with the host cell cytoplasm.

Analysis of length and GC content of the SSU rRNA gene of the microsporidian pathogen

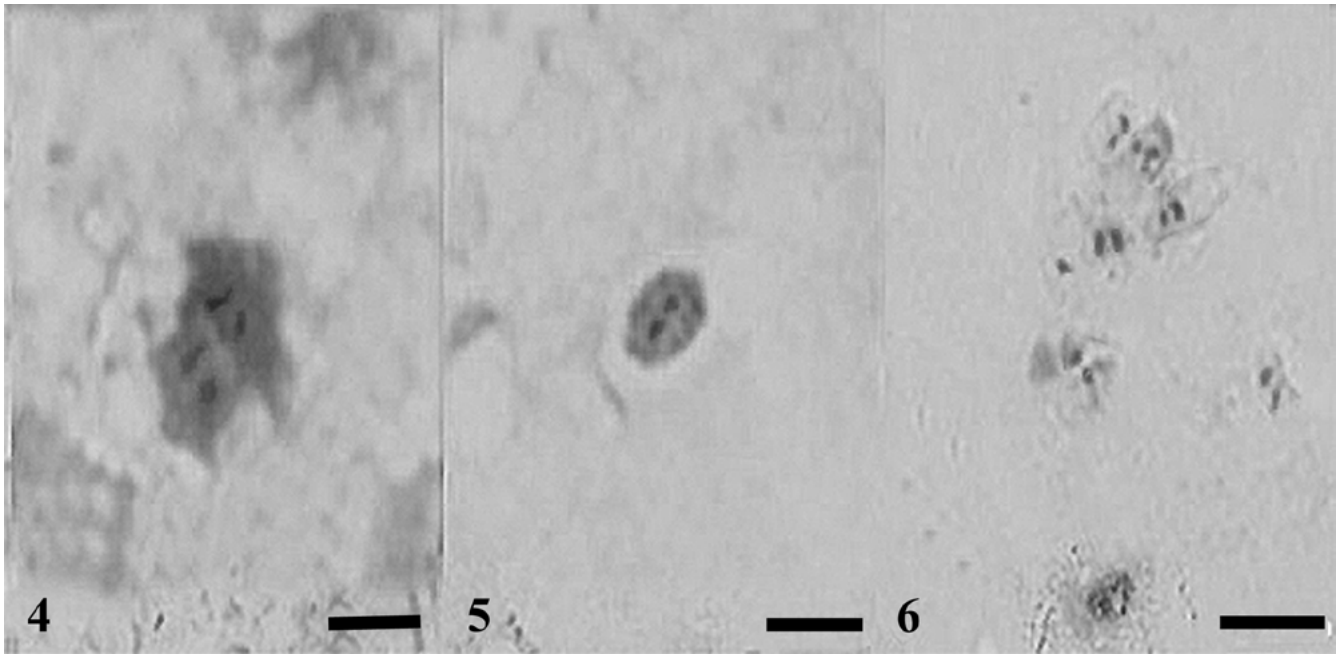
1186 bps were amplified of which 1167 were used in analyses. The GC content of the described microsporidian pathogen was determined as 36.5%.

Phylogenetic analysis

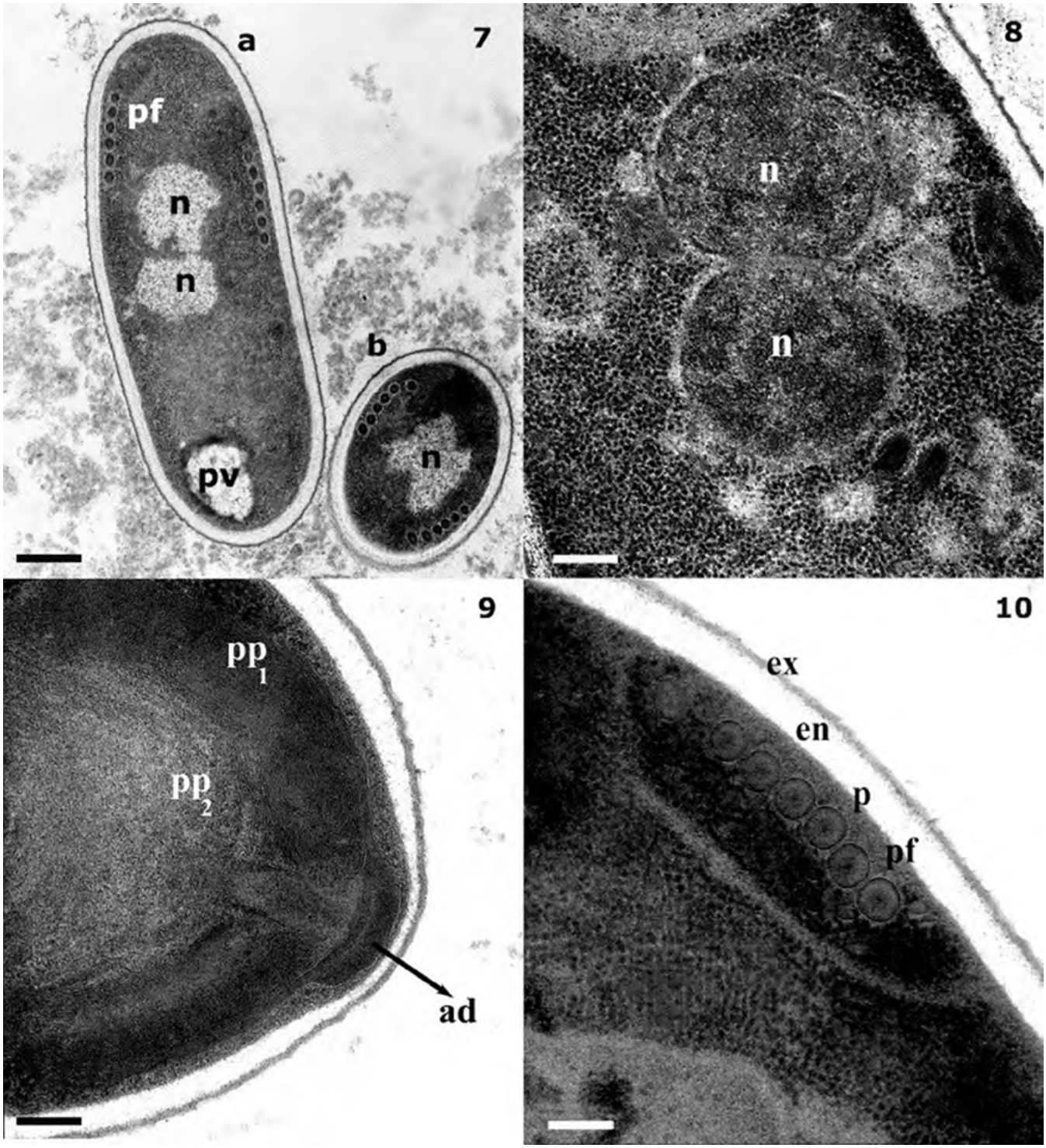
The phylogenetic tree produced 3 major clades; *Endoreticulatus* and *Pleistophora* species were placed in the first group, *Nosema* and *Variomorpha* species, commonly infecting lepidopteran species in the second group, and Nosema and *Variomorpha* species infecting host species from different orders in the third group. According to the phylogenetic tree, our microsporidium separated from other microsporidia species which were detected from *Pieris* species. The novel microsporidian clustered together with *Nosema thomsoni*, a microsporidium from tortricids. Other *Nosema* species isolated so far from pierids according to our phylogeny belonged to another clade. They were separated from each other and settled in different cluster. *Nosema* sp. from *P. rapae* (AF240354) clustered with *N. granulosis* (AJ011833) from *Gammarus duebeni* and uncultured *Nosema* from *P. rapae* (AF485270) with *Nosema* sp. from *Bombyx mori*. *Endoreticulatus* and *Pleistophora* species grouped together and formed a cluster (Fig. 11).

DISCUSSION

The pathogen found in *P. brassicae* is definitely a microsporidium and it belongs to the genus *Nosema*. The spore ultrastructure elucidated the typical characteristics such as a polar filament with an anchoring disc,



Figs 1–6. Light micrographs of the microsporidian pathogen infecting *P. brassicae*. **1** – intestine which is heavily infected with microsporidian spores; **2–3** – microsporidian spores in fresh smears, note that meront and sporoblast stages are easily seen and marked by arrows; **4** – tetranucleate spherical meront (schizont); **5** – binucleate oval meront; **6** – diplokaryotic sporoblast. Scale bars: 30 μm (1), 15–10 μm (2–3), 3 μm (4), 2 μm (5), 4 μm (4).



Figs 7–10. Transmission electron micrographs of microsporidian spores infecting *P. brassicae*. **7** – longitudinal (a) and transversal (b) sections of diplokaryotic spores, polar filament (pf), posterior vacuole (pv) and nuclei (n) are easily seen; **8** – spherical nuclei (n); **9** – polaroplast (pp) and anchoring disc (ad) structures; pp_1 thin lamellar type polaroplast, pp_2 thick lamellar type polaroplast; **10** – cross section of polar filaments; exospore (ex), endospore (en), plasmalemma (p) and polar filament (pf). Scale bars: 800 nm (7), 250 nm (8), 200 nm (9, 10).

a polaroplast, a posterior vacuole and a lack of mitochondria (Figs 7–10) (Larsson 1986, 1988; Canning and Vavra 2000). The light microscopic and ultrastructural studies on the development cycles of the microsporidian pathogen of *Pieris brassicae* and molecular results (SSU rRNA gene with 36.5% GC content) showed that the pathogen belongs to the genus *Nosema* Naegeli, 1857. In insects, 63 different microsporidian genera had been found (Undeen and Vávra 1997) and the genus *Nosema* is one of the biggest groups, including 200 species. These species are mostly pathogenic in invertebrates (Sprague 1982). The genus *Nosema* is frequently a chronic pathogen of both beneficial and harmful insects groups, being prevalent especially in Lepidoptera (Cheung and Wang 1995). Although microsporidia are common pathogens in Lepidoptera, only a few *Pieris* species were shown to be infected with microsporidia (Choi *et al.* 2002). To date some microsporidia have been found to cause natural infections in the cabbage white butterfly species, *Pieris brassicae*, *P. rapae* and *P. canidia* (Malone and McIvor 1996). Paillot (1918) identified the first microsporidian parasite of *P. brassicae* L., *Nosema mesnili*. Later five more microsporidian species were found in *Pieris* species, and only three of them infect *Pieris brassicae* (Table 2). These microsporidian species included the genera *Nosema*, *Endoreticulatus* and *Thelohania*. In contrast to the genera *Endoreticulatus* and *Thelohania*, *Nosema* has diplokaryotic spores (Undeen and Vavra 1997).

Two *Nosema* species, *Nosema mesnili* (Paillot 1918, Tanada 1953, Cheung and Wang 1995) and *Nosema* sp. (Choi *et al.* 2002) are known to infect *Pieris* spp. The *Nosema* species presented here differs from these *Nosema* species in several characteristic features (Table 3).

The number of polar coils and their tilting angles provide very effective taxonomic information for discriminating species (Cheung and Wang 1995). The number of polar coils and their tilting angles of the described pathogen are different from that of *Nosema mesnili* (Cheung and Wang 1995) and *Nosema* sp. (Choi *et al.* 2002). The pathogen presented here has 6 coils with the angle tilt being 25–30°. *N. mesnili* from *P. canidia* has 11 coils with the angle tilt being 10–15° (Cheung and Wang 1995), *N. mesnili* from *P. brassicae* has 11–12 coils with the angle tilt being 40–45° (Sokolova *et al.* 1998) and *Nosema* sp. from *P. rapae* has 12 coils (Choi *et al.* 2002). The new microsporidium has an isofilar polar filament and mature coils measure 57–71 nm in diameter. Furthermore, there is a remarkable difference in spore size between *Nosema mesnili* and the presented microsporidium. The microsporidium presented here is larger (4.21 (3.13–5.47) × 1.91 (1.31–2.45) μm) than *Nosema mesnili isolates* (3–4 × 1.5–2 μm (Paillot 1918), 1.70 × 0.63 μm (Sokolova *et al.* 1998) and 3–4 × 1.5–2 μm (Cheung and Wang 1995)) and the variation in size is higher.

The presented microsporidium with the lamellar region (thin and thick lamellae) has also differences in polaroplast structure from *N. mesnili* isolates (palaroplast with two regions, lamellar region and vascular region (Cheung and Wang 1995) and thin lamellae, thick lamellae and vascular type (Sokolova *et al.* 1998).

Furthermore, the present microsporidium infected only the intestine and all developmental life stages are in direct contact with host cytoplasm, whereas *N. mesnili* also infects the Malpighian tubules, silk glands, fat body and hemocytes. According to Sprague *et al.* (1992) the host species and infection localities are important taxonomic characteristics, at least in microsporidia which infect insects. On the other hand, it is not clear that all *Nosema mesnili* isolates are in the true genus (*Nosema*). Sokolova and Entzeroth (1995) and Sokolova *et al.* (1998) observed pansporoblasts containing octospores in their *N. mesnili* isolates. It is not typical for the genus *Nosema.* Malone and McIvor (1996) proposed that *Nosema mesnili* (Paillot), *Microsporidium* (*Thelohania*) *mesnili* (Paillot) and the New Zealand isolate are identical species and placed in the genus *Vairimorpha*. On the other hand there is lack of information on the SSU rRNA gene sequence of true *N. mesnili* to compare the phylogenetic relationships of different isolates in the literature.

According to our phylogenetic tree, the microsporidium presented in this study differs from other microsporidia species from *Pieris* species and grouped in the same branch with *Nosema thomsoni* (EU219086) recorded from *Choristoneura conflictana* (Lep; Tortricidae). But *N. thomsoni* shows several dissimilarities compared to our microsporidium concerning in host species (*Choristoneura conflictana)* and host family (Tortricidae), site of infection (gut, silk glands and Malpighian tubules), spore dimensions (fresh spores, 1.4 × 2.7 μm; stained spore, 1.2 × 2.4 μm) and locality (Ontario, Canada) (Wilson and Burke 1970) (Table 4). The host species, infection localities and spore dimensions are important taxonomic characteristics, at least in microsporidia which infect insects (Larson 1986, 1988; Sprague *et al.* 1992; Undeen and Vavra 1997; Canning and Vavra 2000).

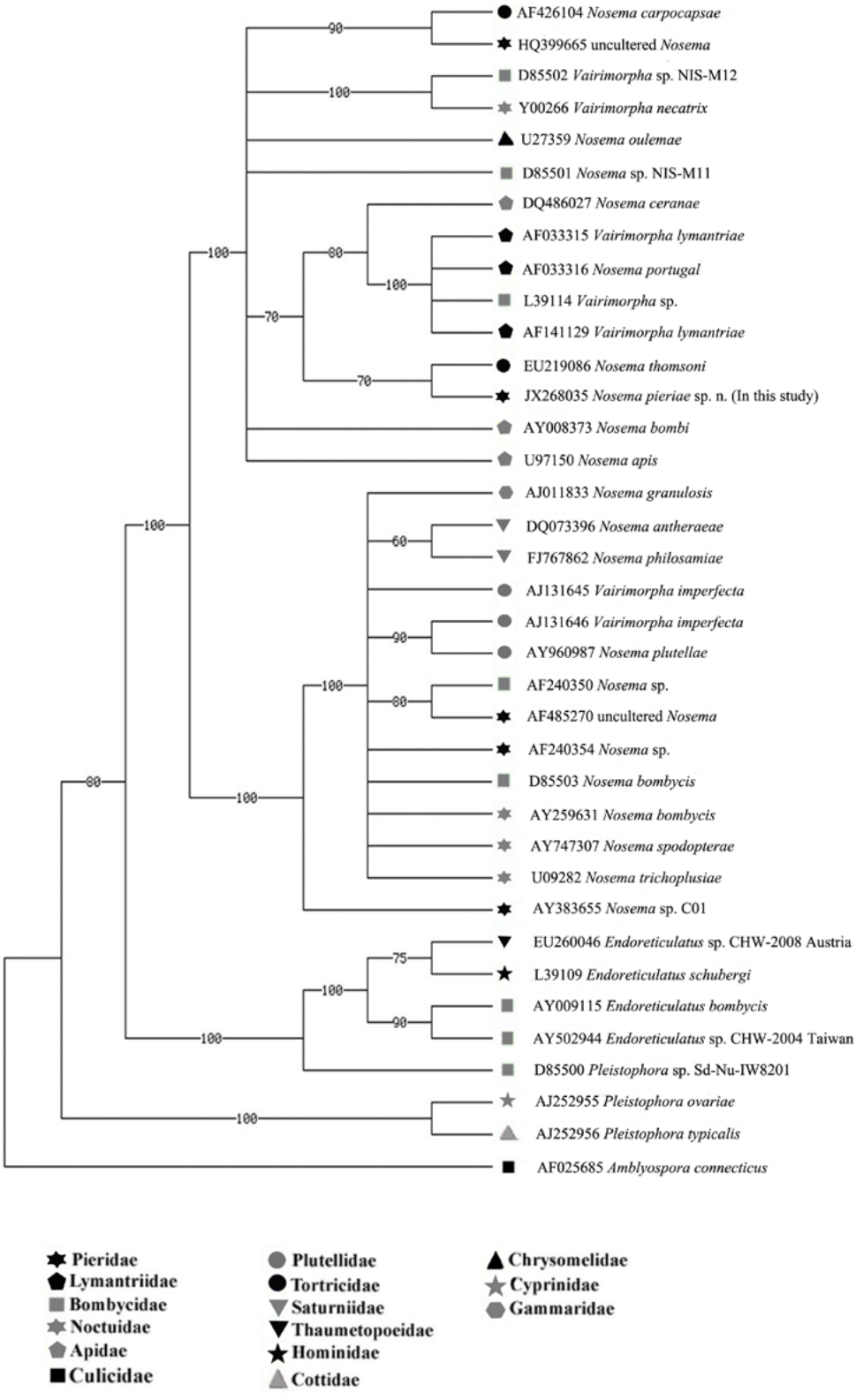


Fig. 11. The phylogenetic analysis was carried out by Maximum Likelihood (ML) using an HKY85 substitution model of PAUP 4.0b10 software. The topology of the consensus tree was constructed and evaluated by 1000 bootstrap replications. The branches with lower than 50% confidence values were ignored.

Table 2. Microsporidia described or reported from *Pieris* spp.

Microsporidian species	Spore size	Host	References
Microsporidium (Thelohania) mesnili	2.5–3.5 × 1.5–2 µm	*P. brassicae*	Paillot (1924)
Pleistophora schubergi aporiae	2 × 1.5 µm	*P. brassicae* *P. rapae*	Veber (1956) Issi (1969)
Thelohania sp.	5.6–6.8 × 3.1–3.8 µm 3.8 × 1.8 µm	*P. rapae*	Laigo and Paschke (1966)
Vairimorpha sp.	3.1 × 1.9 µm (Octosporous) 8.0 × 2.1 µm (Macrosporous)	*P. rapae*	Malone and McIvor (1996)
Nosema (Perezia) mesnili	3–4 × 1.5–2 µm	*P. brassicae*	Paillot (1918)
Nosema sp.	3.8–4.7 × 1.9–2.6 µm	*P. rapae*	Choi *et al*. (2002)

Table 3. Characteristics of *Nosema* species described from *Pieris* spp. (Lepidoptera: Pieridae).

		Nosema mesnili (Cheung and Wang 1995)	*Nosema* sp. (Choi *et al.* 2002).	*Nosema pieriae* sp. n. (Present study)
Host insect		*Pieris brassicae*	*Pieris rapae*	*Pieris brassicae*
Infected organs		Malpighian tubules, silk glands, fat body, hemocytes	Intestine and fat body	Intestine
Spore shape		Oblong or oval	Spherical	Oval
Spore size		3–4 × 1.5–2 µm	3.8–4.7 × 1.9–2.6 µm	4.21 × 1.91 µm
Ultrastructural features	Spore wall thickness	107–138 nm (from illustration)	–	100–125 nm
	Polar filament	11	12	6
	Polar filament diameter	54–69 nm (from illustration)	–	57–71 nm
	Polaroplast	Lamellar-vesicular	–	Lamellar
	Nuclei	Diplokaryotic	Diplokaryotic	Diplokaryotic

Table 4. Characteristics of the new *Nosema* species described from *Pieris brassicae* (Lepidoptera: Pieridae) and *Nosema thomsoni* from *Choristoneura conflictana* (Lepidoptera: Tortricidae).

	Locality	Host	Infection site	Spore shape	Spore size	Reference
Nosema thomsoni	Ontario/Canada	*Choristoneura conflictana*	Gut, silk glands and Malpighian tubules	–	1.0–1.5 × 2.0–2.6 µm	Wilson and Burke 1970
Nosema pieriae sp. n.	Beşikdüzü/Turkey	*Pieris brassicae*	Intestine	Oval	3.13–5.47 × 1.31–2.45 µm	(In this study)

It is evident that the presented microsporidium infecting *P. brassicae* is different from the known species by several features, such as spore type, spore dimension, number of polar filament coils, infected organs, ultrastructural characteristics and phylogeny. Additionally, there is no information on the SSU rRNA gene sequence of true *N. mesnili.* Therefore, the described *Nosema* species from *P. brassicae* seem to be sufficiently distinct to present it as a new species. It is named as *Nosema pieriae* sp. n. after its host genus.

Taxonomic summary

***Nosema pieriae* sp. n.**

Host: *Pieris brassicae* Linneaus (Lepidoptera: Pieridae).

Site of infection: Gut epithelium.

Interface: Meronts and disporous sporonts in direct contact with host cytoplasm.

Spores: Binucleate free spores are oval shaped, mean dimensions: 4.21 × 1.91 µm, n = 50 (range 3.13–5.47 × 1.31–2.45 µm), 6 coils of isofilar polar filament.

Locality: Specimens described here were collected from Beşikdüzü (Trabzon), Turkey.

Deposition of specimens: The samples for light and electron microscopy are preserved in Zoology – II Laboratory, Biology Department at the Karadeniz Technical University, Trabzon – Turkey, with the Catalog No. MÇPB-01.

Etymology: The name of the species refers to the genus name of the host, *Pieris brassicae*.

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REFERENCES

Canning E. U., Vavra J. (2000) Phylum Microsporida. In: The Illustrated Guide to The Protozoa, (Eds. J. J. Lee, G. F. Leedale, P. Bradbury), Allen Press Inc. Lawrence: 39–126

Chen D., Shen Z., Zhu F., Guan R., Hou J., Zhang J., Xu X., Tang X., Xu L. (2012) Phylogenetic characterization of a microsporidium (*Nosema* sp. MPr) isolated from the *Pieris rapae. Parasitol Res.* **111:** 263–269

Cheung W. W. K., Wang J.-B. (1995) Electron microscopic studies on *Nosema mesnili* Paillot (Microsporidia: Nosematidae) infecting the Malpighian tubules of *Pieris canidia* larva. *Protoplasma* **186:** 142–148

Choi J. Y., Kim J. G., Choi Y. C., Goo T. W., Chang J. H., Je Y. H., Kim K. Y. (2002) *Nosema* sp. isolated from Cabbage White Butterfly (*Pieris rapae*) collected in Korea. *J. Microbiol.* **40:** 199–204

Higes M., Martin R., Meana A. (2006) *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. *J. Invertebr. Pathol.* **92:** 93–95

Hylis M., Weiser J., Oborník M., Vávra J. (2005) DNA isolation from museum and type collection slides of microsporidia. *J. Invertebr. Pathol.* **88:** 257–260

Issi I. V. (1969) On possible ways of speciation among Microsporidia. *Prog. Protozool. Proc. Int. Congr. Protozool.* **3:** 337–378

Karnovsky M. J. (1971) Use of ferrocyanide-reduced osmium tetroxide in electron microscopy. *Proc. 14th Ann. Meet. Am. Soc. Cell Biol.*, 146

Laigo F.M. and Paschke J. D. (1966) A microsporidian, *Thelohania* sp., in *Pieris rapae*. *J. Invertebr. Pathol*. **8:** 269–270

Larsson J. I. R. (1986) Ultrastructure, function, and classification of microsporidia. *Progress in Protistology* **1:** 325–390

Larsson J. I. R. (1988) Identification of microsporidian genera (Protozoa, Microspora) – a guide with comments on the taxonomy. *Arch. Protistenkd*. **136:** 1–37

Malone L. A., Mclvor C. A. (1996) Use of nucleotide sequence data to identify a microsporidian pathogen of *Pieris rapae* (Lepidoptera, Pieridae). *J. Invertebr. Pathol.* **68:** 231–238

Paillot A. (1918) Deux microsporidies nouvelles parasites des chenilles de *Pieris brassicae*. *C. R. Soc. Biol.* **81:** 66–68

Paillot A. (1924) Sur *Thelohania mesnili*, microsporidie nouvelle parasite de *Pieris brassicae* L. *C. R. Soc. Biol*. **90:** 501–503

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

Rizvi P. Q., Ali A., Khalid S. (2009) Age and stage-specific life-table of cabbage butterfly *Pieris brassicae* L. (Lepidoptera: Pieridae) on various cole crops. *J. Plant Protect. Res.* **49:** 145–150

Sokolova Y. Y., Timoshenko S. A, Issi I. V. (1988). The morphogenesis and ultrastructure of *Nosema mesnili* Paillot (Microsporidia, Nosematidae). *Tsitologiia* **30:** 26–33

Sokolova Y. Y., Entzeroth R. (1995) Morphological and immunological studies on microsiporidia: Antigene properties of *Nosema mesnili* spores. *Arch. Protistenkd*. **145:** 100–104

Sprague V. (1982) Microspora. In: Synopsis and classification of living organism (Eds. S. P. Parker), McGraw Hill Book Co., New York**,** 589–594

Sprague V., Becnel J. J., Hazard E. I. (1992) Taxonomy of phylum Microspora. *Crit. Rev. Microbiol.* **18:** 285–395

Spurr A. R. (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. *Clin. Microbiol. Res.* **3:** 197–218

Tanada Y. (1953) Description and characteristics of a granulosis virus of the imported cabbageworm. *Proc. Hawaii. Entomol. Soc*. **15:** 235–260

Undeen A. H., Vávra J. (1997) Research methods for entomopathogenic protozoa. In: Manual of Techniques in Insect Pathology. Biological techniques series, (Ed. L. Lacey). Academic Press, London**:** 117–151

Veber J. (1956) *Plistophora aporiae* n. sp., ein parasit des Baumweisslinges *Aporia crataegi*. *Cesk. Parasitol*. **3:** 181–185

Vossbrinck C. R., Baker M. D., Didier E. S., Debrunner-Vossbrinck B. A., Shadduck J. A. (1993) Ribosomal DNA sequences of *Encephalitozoon hellem* and *Encephalitozoon cuniculi*: species identification and phylogenetic construction. *J. Eukaryot. Micorbiol.* **40:** 354–362

Vossbrinck C. R., Maddox J. V., Frideman S., Debrunner-Vossbrinck B. A., Woese C. R. (1987) Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. *Nature* **326:** 411–414

Wilson G. G., Burke J. M. (1970) *Nosema thomsoni* n. sp., a microsporidian from *Choristoneura conflictana* (Lepidoptera: Tortricidae). *Can. J. Zool.* **49:** 786–788

Yaman M., Radek R. (2003) *Nosema chaetocnema* sp. n. (Microspora: Nosematidae), a microsporidian parasite of *Chaetocnema tibialis* (Coleoptera: Chrysomelidae). *Acta Protozool.* **42:** 231–237

Yaman M., Radek R., Toguebaye B. (2008) A new microsporidian of the genus *Nosema*, parasite of *Chaetocnema tibialis* (Coleoptera: Chrysomelidae) from Turkey. *Acta Protozool.* **47:** 279–285

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