Parasitology

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A new isolate of *Nosema fumiferanae* (Microsporidia: Nosematidae) from the date moth *Apomyelois* (*Ectomyelois*) *ceratoniae*, Zeller, 1839 (Lepidoptera: Pyralidae)

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Research Article

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Abstract

In this study, a microsporidian pathogen of the date moth (*Apomyelois* (*Ectomyelois*) *ceratoniae*, Zeller, 1839) also known as the carob moth, is described based on light microscopy, ultrastructural characteristics and comparative molecular analysis. The pathogen infects the gut and hemolymph of *A. ceratoniae*. All development stages are in direct contact with the host cell cytoplasm. Fresh spores with nuclei arranged in a diplokaryon are oval and measured 3.29 ± 0.23 μm (4.18–3.03 μm, $n = 200$) in length and 1.91 ± 0.23 μm (2.98–1.66 μm, $n = 200$) in width. Spores stained with Giemsa's stain measured 3.11 ± 0.31 μm (3.72–2.41 μm, $n = 150$) in length and 1.76 ± 0.23 μm (2.16–1.25 μm, $n = 150$) in width. Spores have an isofilar polar filament with 10-12 coils. An 1110 bp long alignment of the current microsporidium showed an *SSU rRNA* gene difference of only 0.0009, corresponding to >99.91% sequence similarity with *Nosema fumiferanae*, while *RPB1* gene sequences were 98.03% similar within an alignment of 969 bp. All morphological, ultrastructural and molecular features indicate that the microsporidian pathogen of *A. ceratoniae* is the new isolate of the *N. fumiferanae* and is named here as *Nosema fumiferanae* TY61.

Introduction

The date moth [*Apomyelois* (*Ectomyelois*) *ceratoniae*, Zeller], also known as the carob moth is a serious pest of the many fruits from a wide range of plant families as well as dried fruits during storage (Gothilf, 1984; Warner, 1988). This cosmopolite polyphagous pest causes significant damage to various crops throughout the world, which varies by region, host plant and plant variety. For instance, while it causes infestation on the date palm (*Phoenix dactylifera* L.) in Tunisia and Algeria, it is a major pest of the pomegranate (*Punica granatum* L.) in Iran and Turkey (Norouzi *et al*., 2008; Idder *et al*., 2009; Öztürk and Ulusoy, 2009; Zouba *et al*., 2009; Öztop *et al*., 2010). Besides these crops, there are lots of records of its damage on other host plants such as pistachio, *Pistacia vera* L. (Dhouibi, 1982; Mehrnejad, 1993), carob, *Ceratonia siliqua* (Gothilf, 1964), almond, *Prunus dulcis* (Mill.) (Gothilf, 1984), fig, *Ficus carica* L. (Shakeri, 1993), walnut, *Juglans nigra* L. (Balachowsky, 1975), dried fruits and nuts.

The struggle of the date moth is also varied, as are the plant species in which it causes damage. In Iran, this pest is controlled by collecting and burning infected pomegranate fruits at the end of the growing season that reduces overwintering sites (Behdad, 1991). However, the date moth is widely controlled with different pesticides like methyl bromide which is highly toxic and poses several hazards to animals and humans (Hallier *et al*., 1990). Using chemicals against pests affects the environment and non-target organisms negatively, and these effects have led to new approaches, especially in the last quarter, to identify the natural pathogens of pests (Bekircan *et al*., 2017; Bekircan, 2020).

Microsporidia (Opisthokonta) phylum, is a very special group that infect the diverse Animalia taxa, especially Insecta (Solter *et al*., 2012). This phylum has 200 genera and more than 1300 species, it consists of intracellular pathogens that cause various abnormalities on their hosts (Becnel *et al*., 2014). Especially, entomopathogenic microsporidia have detrimental effects on insects including reduced longevity and fecundity (Hajek and Delalibera, 2010). Because of these effects, microsporidia can be used as natural regulators against certain pest insect species.

In this study, the microsporidian pathogens of *A ceratoniae* were investigated and a new isolate of the *Nosema fumiferanae* (*Nosema fumiferanae* TY61) complete description was done for the first time based on morphological and molecular data.

Materials and methods

Insect samples and light microscopy

Apomyelois ceratoniae individuals were collected from October to December 2017–2019 in Trabzon, Turkey. The larvae and adult members, which collected from nuts storages, were placed in separate plastic boxes and transported laboratory as soon as possible. The internal

Table 1. 16S Small subunit (SSU) ribosomal RNA and RNA polymerase II largest subunit (RPB1) gene sequences used for phylogenetic analyses

	Accession No	Organism name	Host	Order	Family
16S SSU rRNA	MN861969	*Nosema fumiferanae* TY61	*Apomyelois* (*Ectomyelois*) *ceratoniae*	Lepidoptera	Pyralidae
	KT020736	*Nosema fumiferanae*	*Choristoneura fumiferana*	Lepidoptera	Tortricidae
	D85503	*Nosema bombycis*	*Bombyx mori*	Lepidoptera	Bombycidae
	U09282	*Nosema trichoplusiae*	*Trichoplusia ni*	Lepidoptera	Noctuidae
	AJ012606	*Nosema tyriae*	*Tyria jacobaeae*	Lepidoptera	Arctiidae
	EU864526	*Nosema antheraeae*	*Antheraea pernyi*	Lepidoptera	Saturniidae
	Y00266	*Vairimorpha necatrix*	*Pseudaletia unipuncta*	Lepidoptera	Noctuidae
	AY958071	*Nosema pyrausta*	*Ostrinia nubilalis*	Lepidoptera	Crambidae
	AY211392	*Nosema spodopterae*	*Spodoptera litura*	Lepidoptera	Noctuidae
	U26532	*Nosema furnacalis*	*Ostrinia nubialis*	Lepidoptera	Crambidae
	AF033315	*Vairimorpha lymantriae*	*Lymantria dispar*	Lepidoptera	Erebidae
	AF033316	*Nosema portugal*	*Lymantria dispar*	Lepidoptera	Erebidae
	AY940656	*Nosema chrysorrhoeae*	*Euproctis chrysorrhoea*	Lepidoptera	Erebidae
	JX268035	*Nosema pieriae*	*Pieris brassicae*	Lepidoptera	Pieridae
	AF426104	*Nosema carpocapsae*	*Cydia pomonella*	Lepidoptera	Tortricidae
	L28973	*Vairimorpha heterosporum*	*Plodia interpunctella*	Lepidoptera	Pyralidae
	AY940659	*Nosema serbica*	*Lymantria monacha*	Lepidoptera	Lymantriidae
	AY009115	*Endoreticulatus bombycis*	*Bombyx mori*	Lepidoptera	Bombycidae
	L39109	*Endoreticulatus schubergi*	*Cholistoneura fumiferana*	Lepidoptera	Tortricidae
RPB1	MT461295	*Nosema fumiferanae* TY61	*Apomyelois (Ectomyelois) ceratoniae*	Lepidoptera	Pyralidae
	HQ457435	*Nosema fumiferanae*	*Choristoneura fumiferana*	Lepidoptera	Tortricidae
	DQ996231	*Nosema bombycis*	*Bombyx mori*	Lepidoptera	Bombycidae
	DQ996234	*Nosema trichoplusiae*	*Trichoplusia ni*	Lepidoptera	Noctuidae
	AJ278948	*Nosema tyriae*	*Tyria jacobaeae*	Lepidoptera	Arctiidae
	AF060234	*Vairimorpha necatrix*	*Pseudaletia unipuncta*	Lepidoptera	Noctuidae
	HQ457438	*Nosema disstriae*	*Malacasoma disstria*	Lepidoptera	Lasiocampidae
	XM 002995356	*Vairimorpha ceranae*	*Apis ceranae*	Hymenoptera	Apidae
	DQ996230	*Vairimorpha apis*	*Apis mellifera*	Hymenoptera	Apidae
	DQ996232	*Nosema empoascae*	*Empoasca fabae*	Homoptera	Cicadellidae
	DQ996233	*Nosema granulosis*	*Gammarus duebeni*	Amphipoda	Gammaridae
	XM 014708712	*Ordospora colligata*	*Daphnia magna*	Cladocera	Daphniidae

organs of thorax and abdomen for each specimen were excised and examined for microsporidiosis by light microscopy according to Yaman *et al.* (2014). Microsporidia positive slides were fixed with methanol for 5 min after air-dried and stained for approximately 10 hours in freshly prepared 5% solution of Giemsa stain (Undeen and Vávra, 1997). Microsporidian spores and life cycle stages were photographed with a Zeiss AXIO microscope combined with Axiocam ERc5s digital camera. Spore measurements were taken using ZEN 2.3 Elements imaging software.

Electron (TEM) microscopy

For transmission electron microscopy (TEM), infected tissues were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1–2 h, washed with cacodylate buffer and postfixed in 1% aqueous OsO_4 for 2 h. After postfixation, the tissues were washed with cacodylate buffer and dehydrated through an ascending alcohol series and acetone before embedding in Spurr's resin (Spurr, 1969; Baki and Bekircan, 2018). Thin sections were taken with Leica EM UC7 ultramicrotome and mounted on Pioloform-coated copper grids which were then stained with saturated uranyl acetate and Reynolds' lead citrate (Reynolds, 1963). The samples were examined and photographed with a HITACHI HT7800 transmission electron microscope.

Molecular studies

Mature spores were obtained from infected tissues which were collected in sterile 1.5 ml Eppendorf tubes and homogenized in Ringer's solution with a micropestle. The suspensions were filtered with cheesecloth and then centrifuged for 2 min at 300 rpm (Chen *et al.*, 2012). Further, 1 mL of distilled water was used to rinse the spore pellet. Purified spores were stored at −20°C until DNA extraction (Martín- Hernández *et al.*, 2007).

Microsporidian DNA was extracted from purified spores using a slightly modified protocol of Higes *et al.* (2006). Purified spores were placed in a 0.5 ml microfuge tube with equal volumes 0.3% hydrogen peroxide (H_2O_2) and kept at room temperature for 15

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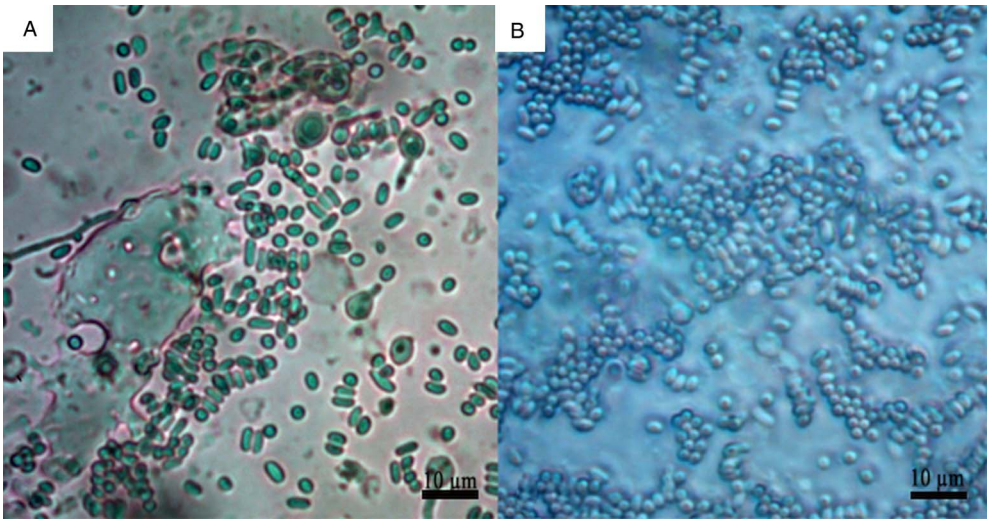

Fig. 1. Light micrographs of spore stages of *Nosema fumiferanae* TY61 from *Apomyelois* (*Ectomyelois*) *ceratoniae*, in wet mount

minutes to stimulate spore wall disruption. An approximately 0.1 g of glass beads (0.425–0.600 μm) were added into the same tube and vigorously shaken for 2 min at maximum speed on the vortex (Hylis *et al.*, 2005). The DNA extraction was then performed with the QIAGEN DNA Isolation Kit, No. 69504 according to the manufacturer's guidelines. To amplify the small subunit rRNA (*SSU rRNA*) and the largest subunit of RNA polymerase II (*RPB1*), the Qiagen Multiplex PCR Kit (QIAGEN, Cat. no. 206143) was used. The 18F/1537R primer set was used to amplify the SSU rRNA gene (18F/1537R: 5′-CACCA GGTTG ATTCT GCC-3′/5′-TTATG ATCCT GCTAA TGGTT C-3′) and the primers for the RPB1 gene were newly designed (Yıldırım and Bekircan, 2020). For sequencing, bidirectional readings were made and after the necessary examinations, a consensus sequence

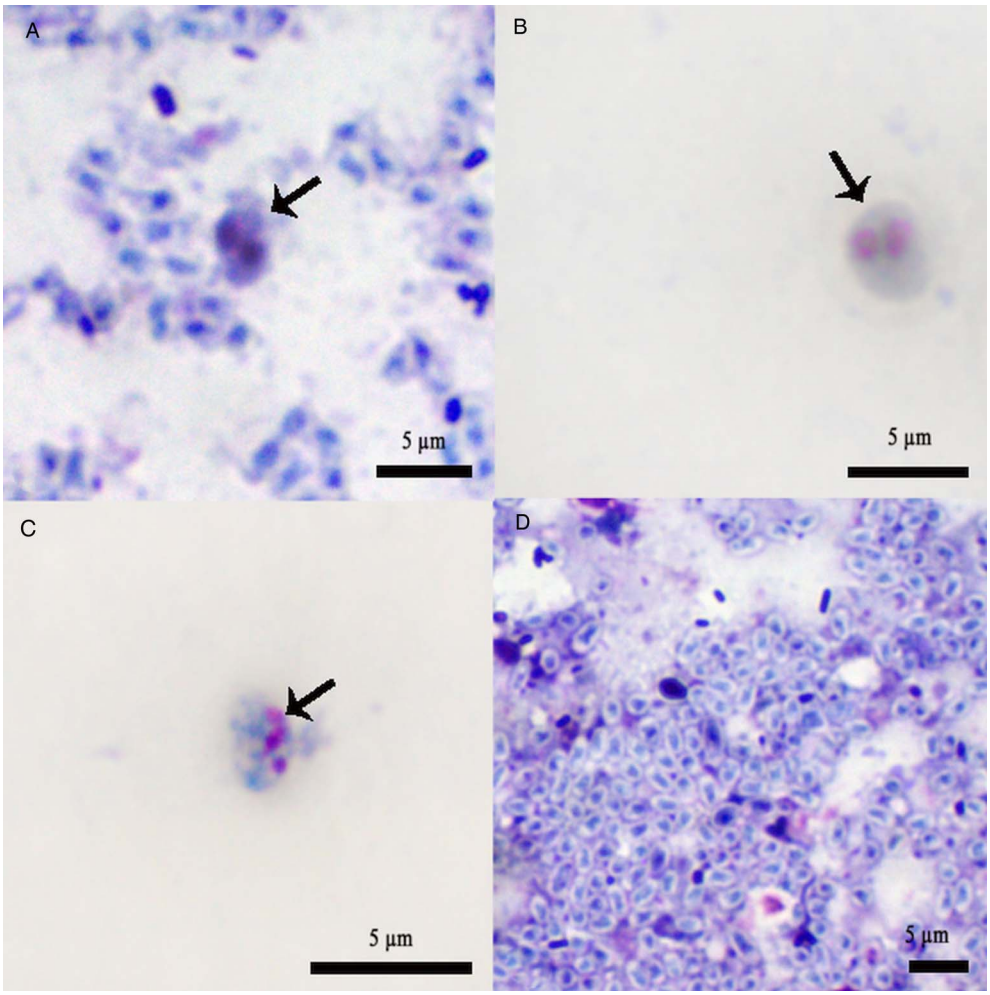

Fig. 2. Light micrographs of the life stages of *Nosema fumiferanae* TY61 from *Apomyelois* (*Ectomyelois*) *ceratoniae*, in Giemsa-stained smears A – diplokaryotic meront (gut); B – diplokaryotic sporonts (gut); C – early sporoblast (gut); D – fresh spores (gut).

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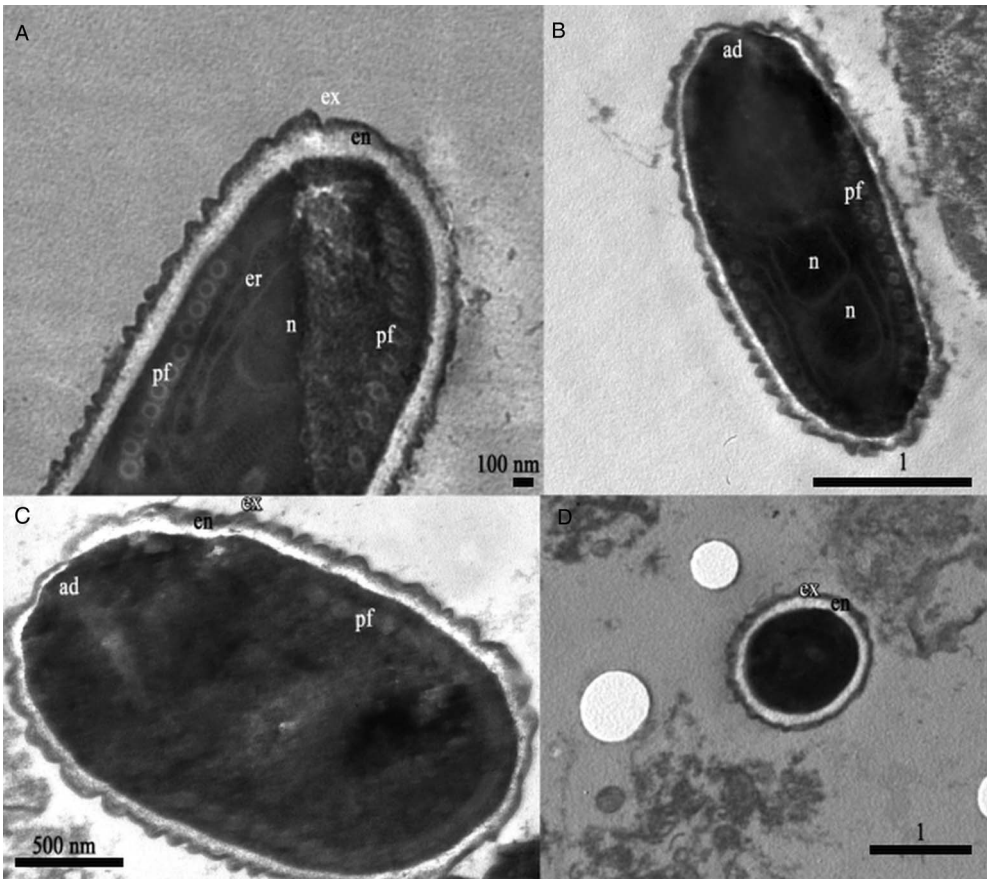

Fig. 3. TEM photographs of *Nosema fumiferanae* TY61 in *Apomyelois* (*Ectomyelois*) *ceratoniae* gut tissue. Diplokaryotic microsporidian spore with a thick wall consisting of a thin exospore (ex) and a thick electron-lucent endospore (en), showing 10–12 coils of the polar filament (pf), a clearly visible diplokaryon (n), regular meshes of endoplasmic reticulum (er) are arranged on both side of diplokaryon and section of the anterior portion of a spore showing an anchoring disc (ad) attenuated apically to the endospore. Bars: A-100nm, B-500nm B, C,D-1 μm.

was established and loaded GenBank. The polymerase chain reaction (PCR) reaction (94°C for 15 min; 45 cycles of 94°C for 30 s; 61°C for 90 s; 72°C for 90 s; and 72°C for 10 min) was processed in a total volume of 50 μL. After the amplification, the 16S SSU rRNA and RPB1 gene base sequences were determined by the Macrogen Inc. Company, The Netherlands.

The microsporidian base sequences were aligned with the closely related species mostly from the genus *Nosema* (Table 1). While *Endoreticulatus bombycis* and *Endoreticulatus schubergi* (Microsporidia: Encephalitozoonidae) were included as outgroup species for 16S *SSU rRNA*, *Ordospora colligata* (Microsporidia: Ordosporidae) were included for *RPB1*. Datasets were aligned using BioEdit and CLUSTAL_W programs. Phylogenetical analyses were performed using either the Maximum Parsimony algorithm with PAUP 4.0a or MEGA 10. The GC content of the base sequences of the current microsporidium and other sequences were analysed with the FastPCR program.

Results

Light microscopy

Between 2017 and 2019, 202 larvae and 45 adults of *A. ceratoniae* were dissected and observed with the light microscope. During the examinations, 19 infected larvae (9.4%) and 7 infected adults (15.5%) were determined (total infection rate 10.5%). Examination by light microscopy showed that the infection was confined to the gut and hemolymph of the host (Fig. 1). Fresh spores were oval in shape and measured 3.29 ± 0.23 μm (4.18–3.03 μm, $n = 200$) in length and 1.91 ± 0.23 μm (2.98–1.66 μm, $n = 200$) in width. During the examinations on Giemsa-stained smears, mature spores and intracellular life stages were observed at the same time. The binucleate spores were in direct contact with the host cell cytoplasm and showed a disporoblastic (*Nosema* type) development. Binucleate meronts are usually spherical and measure 4.30 ± 0.66 μm in diameter ($n = 20$) (Fig. 2A). The spherical binucleate sporonts produced sporoblasts *via* binary fission Spherical sporonts measured 3.30 ± 0.50 μm in diameter (Fig. 2B). Sporoblasts were elongated and measured 5.91 × 3.60 μm (Fig. 2C). After the Giemsa staining, stained mature spores measured as 3.11 ± 0.31 μm (3.72–2.41 μm, $n =$ 150) in length and 1.76 ± 0.23 μm (2.16–1.25 μm, $n = 150$) in width (Fig. 2D).

Electron (TEM) microscopy

The binucleate mature spores were oval in shape (2.85 × 1.43 μm) (Fig. 3). Electron microscopic observations confirmed that the oval spores contained two nuclei in diplokaryotic arrangement with spherical nuclei measuring 375–560 nm in diameter (Fig. 3). The spore wall was thick and measured 106–203 nm, additionally, it had a clear endospore thickness of 64–142 nm and an electron-dense wrinkled exospore thickness of 31–93 nm (Fig. 3). The polar filament was isofilar and had 10–12 polar filament coils (Fig. 3) with a diameter of 80–102 nm. The last coils were immature and hence thinner (Fig. 3). They contained a central core surrounded by four concentric layers (Fig. 3). The developmental stages and spores were in direct contact with the host cell cytoplasm (Fig. 3). A sporophorous vesicle was not observed during the light and electron microscopical observations.

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Table 2. Comparison of current microsporidium and other related microsporidia based on the 16S small subunit ribosomal RNA gene (16S SSU rRNA) and the largest subunit of RNA polymerase II (RPB1) gene by query cover, by nucleotide identity, by Pairwise distance analysis and GC% content.

16S SSU rRNA	MN861969	*Nosema fumiferanae* TY61	Query cover	Per cent identity	Pairwise distances	GC content (33.5%)
	KT020736	*Nosema fumiferanae*	100%	99.91%	0.00090	32.3%
	D85503	*Nosema bombycis*	100%	99.82%	0.00180	34.1%
	U09282	*Nosema trichoplusiae*	100%	99.73%	0.00180	34.1%
	AY958071	*Nosema pyrausta*	100%	99.64%	0.00270	34.1%
	AJ012606	*Nosema tyriae*	100%	99.55%	0.00360	34.4%
	AY211392	*Nosema spodopterae*	100%	99.82%	0.00180	34.1%
	EU864526	*Nosema antheraeae*	100%	99.28%	0.00721	34.3%
	U26532	*Nosema furnacalis*	100%	97.66%	0.02165	33.9%
	Y00266	*Vairimorpha necatrix*	96%	84.54%	0.16465	37.3%
	AF033315	*Vairimorpha lymantriae*	96%	84.45%	0.16534	35.9%
	AF033316	*Nosema portugal*	96%	84.45%	0.16445	35.8%
	AY940656	*Nosema chrysorrhoeae*	96%	84.13%	0.14419	37.5%
	JX268035	*Nosema pieriae*	96%	84.50%	0.16282	36.5%
	AF426104	*Nosema carpocapsae*	96%	84.45%	0.16543	35.3%
	AY009115	*Endoreticulatus bombycis*	10%	90.09%	0.34454	51.3%
	L39109	*Endoreticulatus schubergi*	10%	90.06%	0.34420	51.0%
	L28973	*Vairimorpha heterosporum*	-	-	0.55440	29.7%
	AY940659	*Nosema serbica*	-	-	0.56710	31.9%
RPB1	MT461295	*Nosema fumiferanae* TY61	Query cover	Per cent identity	Pairwise distances	GC content (36.2%)
	HQ457435	*Nosema fumiferanae*	99%	98.03%	0.0243	36.4%
	DQ996234	*Nosema trichoplusiae*	99%	94.30%	0.0629	36.7%
	DQ996231	*Nosema bombycis*	99%	94.30%	0.0628	36.6%
	HQ457438	*Nosema disstriae*	99%	93.16%	0.0764	35.6%
	AJ278948	*Nosema tyriae*	99%	93.06%	0.0638	36.7%
	DQ996233	*Nosema granulosis*	90%	78.09%	0.2906	42.9%
	DQ996232	*Nosema empoascae*	93%	76.82%	0.3227	43.6%
	XM 002995356	*Vairimorpha ceranae*	92%	76.73%	0.3168	31.4%
	AF060234	*Vairimorpha necatrix*	91%	74.94%	0.3654	32.5%
	DQ996230	*Vairimorpha apis*	92%	73.68%	0.3463	31.2%
	XM 014708712	*Ordospora colligata*	85%	71.48%	0.4194	43.3%

'-'No significant similarity found.

Molecular studies

The 16S *SSU rRNA* sequence of the studied microsporidium that was 1110 bp, was deposited in GenBank (MN861969). The GC content of the current microsporidium was 33.5% (for other GC contents see Table 2). Pairwise phylogenetic distances between the current species and other species ranged from 0.0009 to 0.5671. Distances between the current microsporidium and the type species of the genera, *Nosema bombycis* (Nägeli, 1857) and *Vairimorpha necatrix* (Pilley, 1976), were 0.0018 and 0.1646, respectively. The identities of 16S *SSU rRNA* sequences between current microsporidium and other species used in the phylogenetic analysis were 84.13–99.91% (Table 2). According to constructed maximum parsimonic tree, the current microsporidium settled the same branch with *Nosema fumiferanae*, a microsporidium from the Lepidopteran family Tortricidae (Fig. 4).

The *RPB1* gene sequence of the current microsporidium (969 bp) was deposited in GenBank with (MT461295) accession code. Similar parameters that were assessed for the 16S *SSU rRNA* like the GC content, the distances and etc. were analysed too and they were summarized in Table 2. As in the maximum parsimonic tree constructed for 16S *SSU rRNA*, the current microsporidium settled again in the same branch with *N. fumiferanae* in the maximum parsimonic tree which was prepared with *RPB1* base sequences.

An 1110 bp long alignment of the current microsporidium showed an *SSU rRNA* gene difference of only 0.0009, corresponding to >99.91% sequence similarity with *Nosema fumiferanae*, while *RPB1* gene sequences were 98.03% similar within an alignment of 969 bp. These two species were, therefore, very closely related to their biological and morphological features that were evidently similar. Consequently, the phylogenetic status, light

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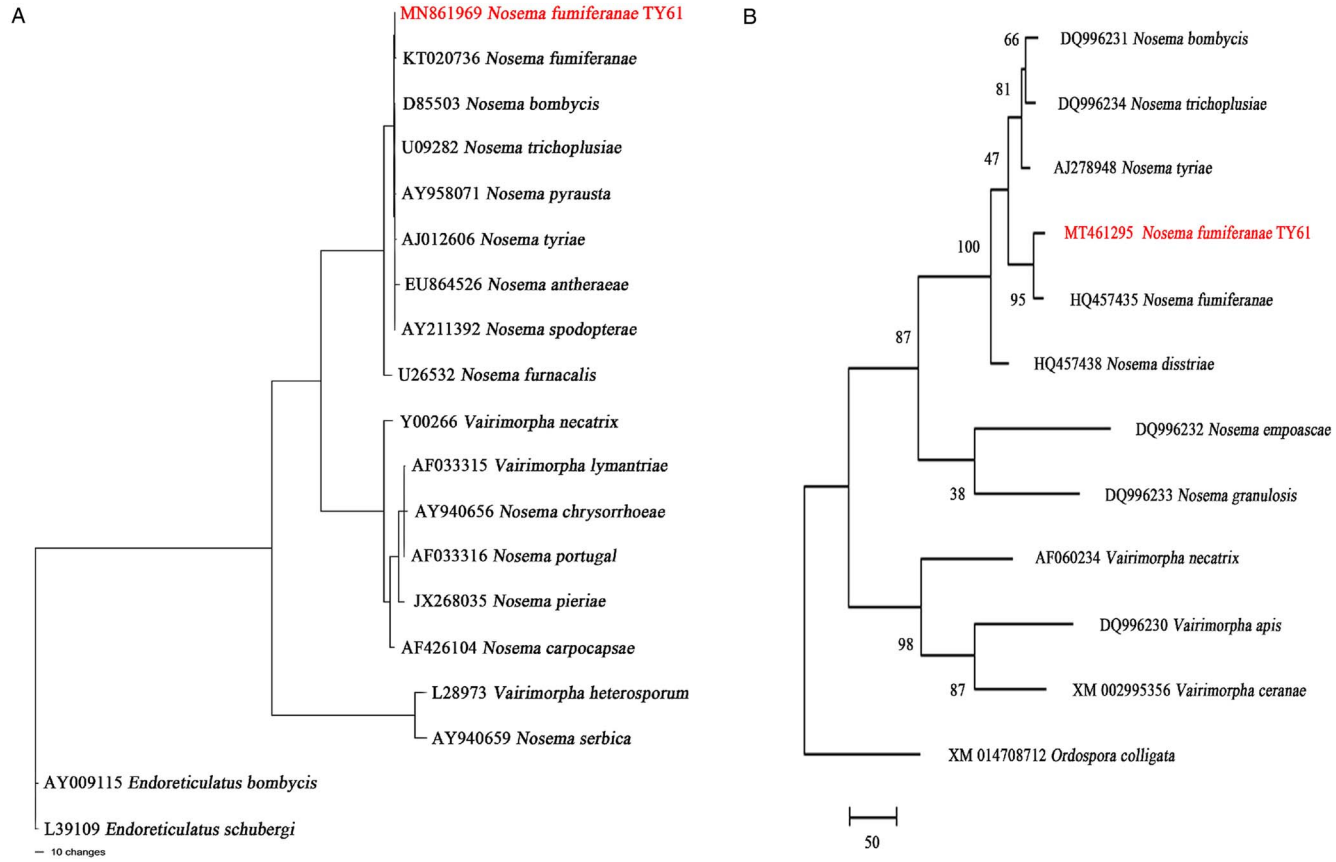

Fig. 4. Phylogeny inferred for *Nosema fumiferanae* TY61 and related taxa with GenBank accession numbers. Tree reconstructions based upon (A) the small subunit rRNA and (B) the largest subunit of RNA polymerase II gene alignments Numbers above the branches are bootstrap support values in percentage.

Table 3. Characteristics of the new *Nosema fumiferanae* isolate described in the present study and other *Nosema fumiferanae* isolates

		Nosema fumiferanae TY61	*Nosema (prezia) fumiferanae*	*Nosema fumiferanae*
Locality		Trabzon, Turkey	Ontario. Canada	Santa Cruz, California
Host		*Apomyelois (Ectomyelois) ceratoniae, Zeller, 1839*	*Chorıstoneura fumıferana*	*Epiphyas postvittana*
Infected organs		Gut and hemolymph	The fat body, silk glands, epidermis, gonads, hindgut and nerve tissue	Malphigian tubules and the silk glands (3 dpi), followed by the hemolymph
Spore shape		Oval	Subcylindrical	Ellipsoidal
Spore size		3.29 ± 0.23 × 1.91 ± 0.23 μm	3–5 × 2 μm	3.8 ± 0.1 × 1.9 ± 0.01 μm
Ultrastructural features	Spore wall thickness	106–203 nm		60–160 nm
	Polar filament	Isofilar 10–12 coils		Isofilar 12–15 coils
	Polar filament diameter	80–102 nm		80-90 nm
	Polaroplast	Lamellar		Lamellar
	Nuclei	Binucleate	Binucleate	Binucleate
References		In this study	Thomson (1955)	Hopper *et al.* (2016)

and electron microscopical observations showed that the microsporidian pathogen of *A. ceratoniae* is the new isolate of the *Nosema fumiferanae*.

Discussion

The date moth [*Apomyelois* (*Ectomyelois*) *ceratoniae*, Zeller] is a cosmopolite pest of the many fruits, nuts and dried fruits during storage (Gothilf, 1984; Warner, 1988). Therefore, numerous studies have been conducted in different parts of the world to determine the organisms that can be used in the control of this insect (Alrubeai, 1988; Elsayed and Bazaid, 2011; Mnif *et al.*, 2013). Similarly, in the study, conducted by Lange in 1991 from Argentina, they declared the microsporidiosis from *A. ceratoniae* which were collected from walnuts. Although it was stated that the microsporidium isolated in this study belong to the genus

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Nosema, no definition could be made at the species level. The determined *Nosema* sp. was identified *via* light and electron microscopy in this study. There are obvious similarities between the taxonomic characters examined in Lange's study and the current research. For instance, fresh spore shape, dimensions, disporoblastic (*Nosema* type) development, electron-dense wrinkled exospore, etc. characters determined as nearly the same in both of the two studies. While the fresh spore dimension of the *Nosema* species presented in here $3.29 \pm 0.23\,\mu m \times 1.91 \pm 0.23\,\mu m$, the Lange's record was $3.7 \pm 0.01\,\mu m \times 1.3 \pm 0.006\,\mu m$. The disporoblastic life cycle was determined in both studies. Also, microsporidia were detected in both studies in direct contact with the host cell cytoplasm. The number of polar coils provides very effective taxonomic information for discriminating microsporidia species (Cheung and Wang, 1995). The current microsporidium has an isofilar 10-12 polar filament coils and mature coils measure 80–102 nm in diameter. Similarly, Lange reported 9-12 polar filament coils from the isolated microsporidium. Unfortunately, there was no molecular data for identifying the Nosema species that was determined by Lange (1991).

In South Africa, Lloyd and friends reported the second microsporidiosis from the *A. ceratoniae* in 2017. Although this study mentioned the presence of microsporidial spores with similar size and morphological characteristics as those reported previously by Lange, there was no data or figure in this study put forward to demonstrate this. On the other hand, in this study, molecular data were available in contrast to the study of Lange. In this study, researchers mentioned that approximately 1148 bp SSU sequence was amplified and according to their BLAST search the isolate showed 99% similarity with the *Nosema carpocapsae* (AF426104) and *Nosema oulemae* (U27359) sequences. Also, their phylogenetic analysis revealed that this isolate clustered with the *Nosema/Vairimorpha* group rather than the 'true' *Nosema* group. Despite revealing such important data and making phylogenetic determinations, there were not any SSU base sequences or GenBank accession codes related to this study. Therefore, it was not possible to phylogenetically compare the current microsporidium with this isolate. The *Nosema* genus has been recently suggested for a new classification by researchers and with this perspective, the RPB1 gene sequence was determined in addition to the 16S rRNA sequence in this study (Tokarev *et al.*, 2020).

According to the phylogenetic tree, the microsporidium presented in this study grouped in the same branch with *Nosema fumiferanae* (KT020736) reported from *Choristoneura fumiferana* Clemens (Lepidoptera: Torticidae) in the 'true' *Nosema* group (Thomson, 1955). Although host species and tissue specificity have historically been important taxonomic characteristics in microsporidia, last researches show that some microsporidian species easily switch hosts among different families (Sprague *et al.*, 2008; Tokarev *et al.*, 2020). Given this situation, it is quite likely that the existing microsporidium isolated from *A ceratoniae* is *N. fumiferanae*. The *N. fumiferanae* isolate described by Thomson in 1955 from Ontario, Canada, have several similarities compared to the current microsporidium concerning in site of infection (most tissues, esp. midgut, fat body), spore dimension (fixed mature spore 3-4 μm, fresh mature spore 3–5 μm) (Table 3). The second study that was conducted for identifying *Nosema fumiferanae* by Hopper *et al.*, in 2016, clarified the ultrastructural features of the *N. fumiferanae*. The ultrastructural characteristics of spore structure, especially polar filament structure, are important parameters for the comparison of microsporidian species (Canning and Vávra, 2000; Becnel *et al.*, 2002; Ovcharenko *et al.*, 2013). While the current microsporidium polar filament number is 10–12 coils (80-102 nm diameter); *N. fumiferanae* has 12–15 coils (80-90 nm diameter). And the spore wall thickness of the current microsporidium is (106–203 nm) thicker than *N. fumiferanae* (60–160 nm) (Table 3). All these diagnostic features are important taxonomic characteristics in microsporidia systematics (Larsson, 1986, 1988; Undeen and Vavra, 1997; Canning and Vavra, 2000). And in all these comparisons between the current microsporidium and the isolate of Hopper *et al.*, it was observed that the measurement and taxonomic characters were overlaps.

In conclusion, the phylogenetic status, light and electron microscopy observations suggest that the described *Nosema* species from *Apomyelois* (*Ectomyelois*) *ceratoniae* is a new isolate of the *Nosema fumiferanae*. We named it as *Nosema fumiferanae* TY61. This work is the first study that confirmed *Apomyelois* (*Ectomyelois*) *ceratoniae* as a host of the *Nosema fumiferanae* isolate.

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