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ORIGINAL PAPER

Description and phylogeny of a new microsporidium from the elm leaf beetle, *Xanthogaleruca luteola* Muller, 1766 (Coleoptera: Chrysomelidae)

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Abstract This study describes a new genus and species of microsporidia which is a pathogen of the elm leaf beetle, *Xanthogaleruca luteola* Muller, 1776 (Coleoptera: Chrysomelidae). The beetles were collected from Istanbul in Turkey. All developmental stages are uninucleate and in direct contact with the host cell cytoplasm. Giemsa-stained mature spores are oval in shape and measured 3.40 ± 0.37 μm in length and 1.63 ± 0.20 μm in width. These uninucleate spores have an isofilar polar filament with 11 turns. The spore wall was trilaminar (75 to 115 nm) with a rugose, electron-dense exospore (34 to 45 nm) and a thickened, electron-lucent endospore (65 to 80 nm) overlaying the plasmalemma. Morphological, ultrastructural, and molecular features indicate that the described microsporidium is dissimilar to all known microsporidian taxa and confirm that it has different taxonomic characters than other microsporidia infecting *X. luteola* and is named here as *Rugispora istanbulensis* n. gen., n. sp.

Keywords Coleoptera · Elm · Microsporidia · Pathogen · Ultrastructure

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Introduction

Microsporidia are obligate intracellular pathogens of most of the insects, as well as other invertebrates and vertebrates (Solter et al. 2012). This monophyletic taxon approximately have 200 genera and more than 1300 microsporidia species (Becnel et al. 2014). Although they mostly have nonlethal detrimental effects on their hosts like reduced longevity or fecundity, some microsporidia are considerable natural regulators against certain insect pest species (Hajek and Delalibera 2010).

The elm leaf beetle, *Xanthogaleruca luteola* (Muller) (Coleoptera: Chrysomelidae), is widespread and detrimental insect pest which adversely affects urban and suburban elms in many countries like Turkey (Wu et al. 1991). In recent years, studies have focused on determining the natural pathogens of pest insects like the elm leaf beetle and in this study, the microsporidian pathogens of *X. luteola* were investigated for described a new microsporidian pathogen of *X. luteola*.

Materials and methods

Host Samples

Larvae and adults of *X. luteola* were collected from April to September 2013–2015 in Istanbul, Turkey. The beetles were put into plastic boxes, transported to the laboratory, and dissected as soon as possible.

Light microscopy

The beetles were dissected in Ringer's solution and smeared on microscopic slides then observed under a light microscope at magnifications of 400× to 1000× for detection of

microsporidian pathogens (Yaman et al. 2014). Smears, with microsporidia, were air-dried and fixed in methanol for 10 min. Later, they were stained with Giemsa stain (Undeen and Vávra 1997). Microsporidian spores were photographed with a Leica DM1000 microscope combined with Leica ICC50 digital camera. Spore measurements were taken using LAS EZ 1.0 Soft Imaging System imaging software.

Transmission electron microscopy

An infected beetle's tissues were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1–2 h. Then, they were washed with cacodylate buffer and postfixed in 1% aqueous OsO_4 for 2 h. After the end of postfixation, tissues were washed with cacodylate buffer and dehydrated which was performed through an ascending alcohol series into acetone before embedding in Spurr's resin (Spurr 1969). Thin sections were taken with a 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). Prepared samples were examined and digitally photographed under a JEOL JEM 1220 transmission electron microscope.

DNA isolation, amplification, cloning, and sequencing

Microsporidian spores, obtained from the infected tissue at the end of dissection, and glass beads (0.425–0.600 μm) were added in the same 1.5 ml Eppendorf tube and the tube was strongly 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 min 3 h. After the incubation, DNA extraction was performed with the QIAGEN DNA Isolation Kit, No. 69504 according to the manufacturer's guidelines. After the extraction, 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 for amplifying the microsporidial small subunit (SSU) ribosomal ribonucleic acid (rRNA) genes (Vossbrinck et al. 1987, 1993; 2005). The amplification was performed under the kit manufacturer's guidelines. The PCR products were loaded on 1% agarose gel which was supplemented with ethidium bromide (EtBr) for the check. PCR products were cloned into pGEM®-T easy vector systems and then 16S rRNA gene sequences were determined with an Applied Biosystems model 373A DNA sequencer by using the ABI PRISM cycle sequencing kit in Macrogen Inc. Company, Netherlands.

Phylogenetic analysis

In this study, 25 microsporidia sequences were used for phylogenetic analyses. Among those, one sequence was obtained from the present study and while 15 sequences were selected from the NCBI Genbank database according to basic local alignment search tool (BLAST) search, the others were selected from the literature (for accession numbers and host species see Table 1). In addition, *Endoreticulatus* sp. CHW-2008 Austria, the pathogen of *Thaumetopoea processionea* (Lepidoptera: Thaumetopoeidae), and *Endoreticulatus bombycis*, the pathogen of *Bombyx mori* (Lepidoptera: Bombycidae), were used as an out-group in analyses. BioEdit and CLUSTAL_W programs were used for sequence editing and multiple alignments (Hall 1999). The resultant alignment was analyzed by maximum likelihood (ML) algorithm with PAUP*, version 4.0a147 for 32-bit Microsoft Windows.

The model selected as the best fit for 16S rRNA data set was the HKY85+G model by using an automated model selection interface in PAUP program. The phylogenetic tree was concluded with the maximum likelihood method. A maximum likelihood tree was obtained using Kimura 2-parameter model as the nucleotide acid substitution model (Kimura 1980). The nearest neighbor interchange was used as the maximum likelihood heuristic method. The gamma distributed with invariant site rate was considered, and the number of discrete gamma categories was five. Bootstrap analysis based on 1000 replicates was also conducted in order to obtain confidence levels for the branches (Felsenstein 1985).

Finally, the GC content of the SSU rRNA sequence of novel microsporidium and other similar species sequences were analyzed with the FastPCR program (Yaman et al. 2014).

Results

Light microscopy

During the present study, 1029 *X. luteola* beetles were dissected and examined with the light microscope. Two hundred eighty nine of the 1029 beetles were infected with that microsporidium (infection rate 27.89%). According to light microscopic observation, infections were restricted to the host's intestine. During the examinations, mature spores and intracellular stages were observed at the same time. Fresh spores were oval in shape and measured 4.25 ± 0.52 μm (3.3–6.35; $n = 500$) in length and 2.12 ± 0.25 μm (1.45–2.91; $n = 500$) in width (Fig. 1). After the Giemsa staining, life stages of the pathogen were observed in the Giemsa-stained smears (Fig. 2). Uninucleate meronts were spherical and measured 5.14 ± 1.29 μm (2.61–8.36; $n = 30$) × 4.89 ± 0.87 μm (2.83–6.57; $n = 30$); binucleate meronts measured 7.18 ± 1.54 μm

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

Accession no	Organism name	Host	Order	Family
AF426104	*Nosema carpocapsae*	*Cydia pomonella*	Lepidoptera	Tortricidae
D85503	*Nosema bombycis*	*Bombyx mori*	Lepidoptera	Bombycidae
U11047	*Nosema vespula*	*Vespula germanica*	Hymenoptera	Vespidae
AF033316	Nosema portugal	*Lymantria dispar*	Lepidoptera	Erebidae
JX268035	*Nosema pieriae*	*Pieris brassicae*	Lepidoptera	Pieridae
EU219086	*Nosema thomsoni*	*Choristoneura conflictana*	Lepidoptera	Tortricidae
KC596023	*Nosema thomsoni*	*Choristoneura conflictana*	Lepidoptera	Tortricidae
Y00266	*Vairimorpha necatrix*	*Pseudaletia unipuncta*	Lepidoptera	Noctuidae
DQ996241	*Vairimorpha necatrix*	*Pseudaletia unipuncta*	Lepidoptera	Noctuidae
U27359	*Nosema oulemae*	*Oulema melanopus*	Coleoptera	Chrysomelidae
AY008373	*Nosema bombi*	*Bombus agrorum*	Hymenoptera	Apidae
AF495379	*Oligosporidium occidentalis*	*Metaseiulus occidentalis*	Acari	Phytoseiidae
EU260046	*Endoreticulatus sp.* CHW-2008 Austria	*Thaumetopoea processionea*	Lepidoptera	Thaumetopoeidae
U26534	*Nosema apis*	*Apis mellifera*	Hymenoptera	Apidae
DQ235446	*Nosema apis*	*Apis mellifera*	Hymenoptera	Apidae
U11051	*Nosema necatrix*	*Pseudaletia unipuncta*	Lepidoptera	Noctuidae
AF141129	*Vairimorpha lymantriae*	*Lymantria dispar*	Lepidoptera	Erebidae
AF033315	*Vairimorpha lymantriae*	*Lymantria dispar*	Lepidoptera	Erebidae
HM216911	*Anncaliia algerae*	*Homo sapiens*	Primates	Hominidae
AY894423	*Anncaliia meligethi*	*Meligethes aeneus*	Coleoptera	Nitidulidae
EF564602	*Ovavesicula popilliae*	*Popillia japonica*	Coleoptera	Scarabaeidae
AY009115	*Endoreticulatus bombycis*	*Bombyx mori*	Lepidoptera	Bombycidae
U26532	*Nosema furnacalis*	Ostrinia nubialis	Lepidoptera	Crambidae
U09282	*Nosema trichoplusiae*	Trichoplusia ni	Lepidoptera	Noctuidae
KR704648	***Rugispora istanbulensis n. gen., n. sp.***	***Xanthogaleruca luteola***	**Coleoptera**	**Chrysomelidae**

(5.05–11.01; $n = 23$) × 6.25 ± 1.87 μm (3.78–10.83; $n = 23$) in size (Fig. 2b, c). Sporonts were oval and measured 3.88 ± 0.53 μm (2.88–5.43; $n = 48$) × 1.66 ± 0.35 μm (1.09–2.52; $n = 48$) (Fig. 2e). Uninucleate sporonts produced sporoblasts that divided once (disporoblastic) to produce mature spores. Sporoblasts were elongated and measured 5.70 ± 1.16 μm (4.11–9.35; $n = 38$) × 2.25 ± 0.47 μm (1.54–4.43; $n = 38$) (Fig. 2f). After the Giemsa staining, stained spores measured 3.40 ± 0.37 μm (2.50–4.17; $n = 970$) in length and 1.63 ± 0.20 μm (0.92–2.51; $n = 970$) in width (Fig. 2a).

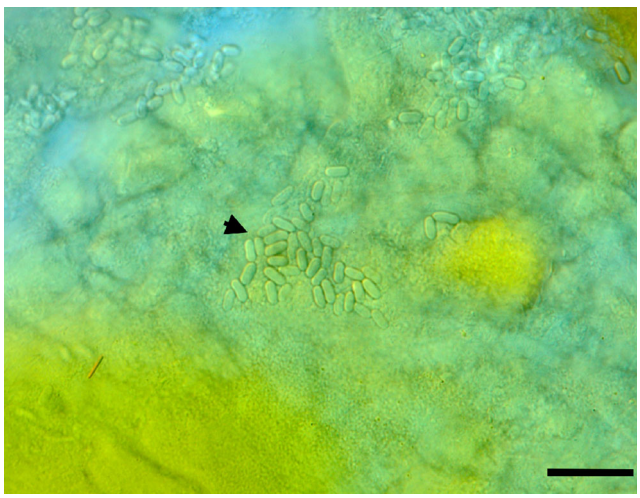

Fig. 1 Light micrograph of *X. luteola* intestine which is heavily infected with *R. istanbulensis* n. gen., n. sp. spores. Scale bar 15 μm

Transmission electron microscopy (TEM)

According to transmission electron microscopy, this novel microsporidium was unikaryotic and occurred in direct contact with the host cell cytoplasm. Merogony appeared to progress from uninucleate to binucleate with karyokinesis (Fig. 3a). Merogony finished with thickening of plasmalemma, and sporogony started. Uninucleate sporonts were mostly spherical in shape (Fig. 3b). During the observation, sporoblasts were characterized with the formation of

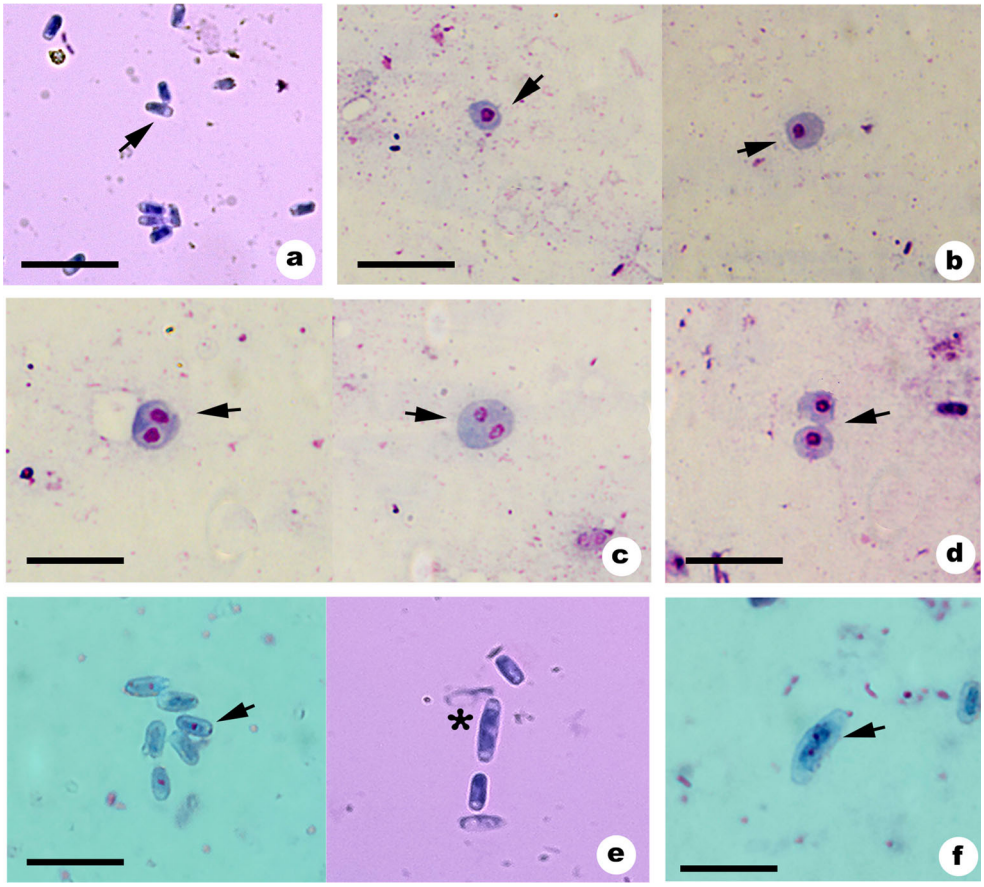

Fig. 2 Light microscopy of the Giemsa-stained slides. **a** Adult spores; **b** uninucleate meronts, scale bar 15 μm. **c** Binucleate meronts; **d** meront undergoing cytokinesis, scale bar 10 μm. **e** Sporonts note that the *asterisk* indicates disporoblastic sporont, scale bar 10 μm. **f** Binucleate sporoblasts before cytokinesis, scale bar 10 μm

primordial polar tubes (Fig. 3b, c). Mature spores were oval in shape and the spore wall was relatively thin (75 to 115 nm); the wrinkled exospore thickness was 34 to 45 nm and the endospore thickness was 65 to 80 nm (Fig. 3). In addition, the spore wall thickness of the anterior apex was very thin compared to other parts (15 to 20 nm) (Fig. 3g). The polar filament was isofilar and had eleven coils. Mature coils measured 67–79 nm in diameter (Fig. 3d). The polaroplast was of the lamellar type occupying the anterior third of the spore (Fig. 3i).

Molecular studies

The novel microsporidian sequence that was 1245 bp was deposited in GenBank with KR704648 accession code. In the analysis, 16S SSU rRNA amplicon ~1245 bp of which 1130 bp were used. According to BLAST analysis, the identity score (ID %) of the novel sequence with the other microsporidian sequences was between 77.0 and 97.0% (Table 2, column 4). Pairwise phylogenetic distances between the novel species and other species varied from 0.0296 to 0.6044 (Table 2, column 5). When compared, the novel microsporidium with the out-group, the phylogenetic distances were determined to be 0.4222–0.4279. The GC content of the novel microsporidian pathogen was determined to be 36.95%. Similarly, the GC contents were determined between 33.93 and 38.69% in *Nosema*, 35.38–37.38% in *Vairimorpha*, 46.91–47.07% in *Anncaliia*, and 51.27–51.30% in *Endoreticulatus* species (for other GC contents see Table 2, column 6). The phylogenetic tree based on the maximum likelihood algorithm showed that the current microsporidium settled on a separate branch within a clade containing the *Nosema*, *Vairimorpha*, and *Oligosporidium* genera (Fig. 4). However, when compared, current microsporidium with the type species belonging to these genera according to the phylogenetic distances, it is away from *Nosema bombycis* with 0.1940, *Vairimorpha necatrix* 0.0405–0.0415, and *Oligosporidium occidentalis* 0.0527. Consequently, the phylogenetic status, light and electron microscopical observations led us to suggest that the microsporidian pathogen of *X. luteola* is the type species of a novel genus. And, we name the pathogen *Rugispora istanbulensis* n. gen., n. sp.

Taxonomic description

Phylum Microsporidia (Balbiani 1882), Unikaryonidae (Sprague 1977).

Genus *Rugispora* n.gen

Presporulation stages and spores are unikaryotic and only one sporulation sequence observed. All stages are in direct

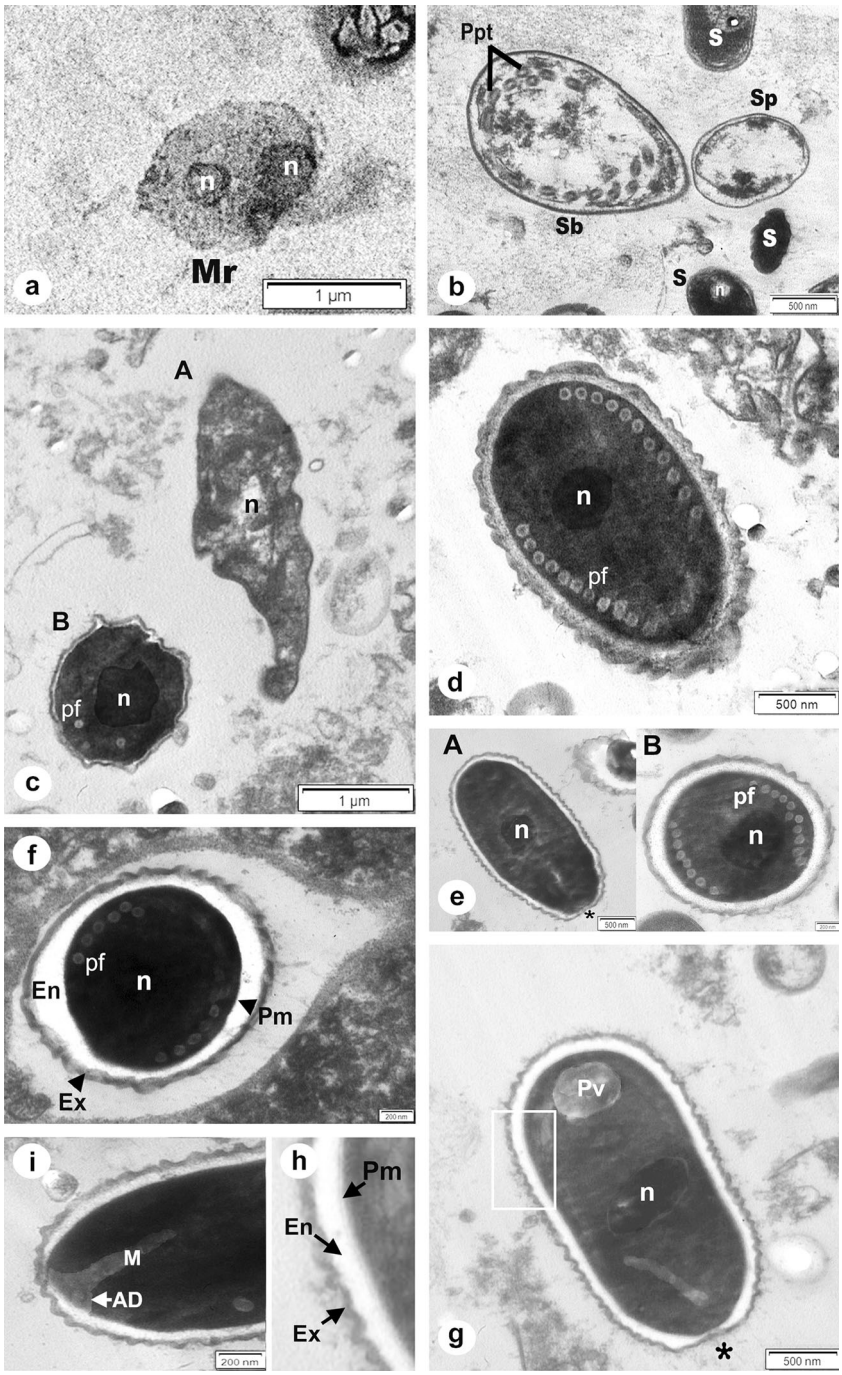

Fig. 3 Transmission electron micrographs of *R. istanbulensis* n. gen., n. sp. **a** Binucleate meront developing in direct contact with the host cell cytoplasm. **b** Spherical sporont and early sporoblast with primordial polar tubes. **c** A, sporoblast stages. B, late sporoblast stages even young spores. **d:** Mature spore with isofilar polar filaments. **e** Longitudinal (*A*) and cross (*B*) section of mature spores. **f** Cross-section of mature spore. **g** Longitudinal section of mature spore, note that anterior apex is thin and flattened indicated with an asterisk. **h** Trilaminar structure of spore wall. **i** Longitudinal section through the anterior portion of a mature spore. Anchoring disk attached to the manubroid portion of polarfilament (M). Abbreviations: *Ad* anchoring disk, *En* endospore, *Ex* exospore, *M* manubrium, *Mr* meront, *n* nucleus, *Pf* polar filament, *Pm* plasma membrane, *Ppt* primordial polar tube, *Pv* posterior vacuole, *S* adult spore, *Sb* sporoblast, *Sp* sporont

contact with the cytoplasm of host midgut cells. Merogony is primarily by binary fission. Sporogony is disporoblastic to produce uninucleate spores characterized by a rugose exospore, mainly parasites of terrestrial invertebrates. The 16S rDNA sequence of the type species *R. istanbulensis* (Acc. No. KR704648) serves as the reference sequence for the genus.

Type species: *Rugispora istanbulensis* n. gen., n. sp.

With the characteristic of the genus, mature spores are oval in shape with average length 3.40 ± 0.37 μm and width 1.63 ± 0.20 μm (fixed, $n = 970$). Fresh spores measured 4.25 ± 0.52 μm (3.3–6.35; $n = 500$) in length and 2.12 ± 0.25 μm (1.45–2.91; $n = 500$) in width. Mature spores contained an isofilar polar filament that made approximately 11 turns. The spore wall was trilaminar with an unlayered and rugose (wrinkled) electron-dense exospore and a thickened, electron-lucent endospore overlaying the plasmalemma. The anterior region of the spore possessed a smooth, thin anterior apex in the region of the anchoring disk. Polaroplast is of the lamellar type with a small but prominent posterior vacuole.

Table 2 Comparison of new microsporidium and other 24 related microsporidia based on the small subunit ribosomal RNA gene by nucleotide identity [first column], by the Kimura 2-parameter distance analysis [second column] and GC% content [third column]

KR704648	*Rugispora istanbulensis* n. gen., n. sp.	Query cover	Percent identity	Kimura 2-parameter distance	GC content (36.95%)
AF426104	*Nosema carpocapsae*	99.0%	97.0%	0.0296	35.24%
D85503	*Nosema bombycis*	99.0%	83.0%	0.1940	34.09%
U11047	*Nosema vespula.*	100%	97.0%	0.0296	36.79%
AF033316	*Nosema portugal*	100%	97.0%	0.0314	35.75%
JX268035	*Nosema pieriae .*	93.0%	96.0%	0.0306	36.50%
EU219086	*Nosema thomsoni*	100%	97.0%	0.0296	36.88%
KC596023	*Nosema thomsoni*	99.0%	97.0%	0.0305	36.95%
Y00266	*Vairimorpha necatrix*	100%	95.0%	0.0415	37.38%
DQ996241	*Vairimorpha necatrix*	100%	96.0%	0.0405	37.05%
U27359	*Nosema oulemae*	100%	96.0%	0.0341	36.85%
AY008373	*Nosema bombi*	100%	95.0%	0.0479	38.09%
AF495379	*Oligosporidium occidentalis*	99.0%	95.0%	0.0527	38.39%
EU260046	*Endoreticulatus sp. CHW-2008 Austria*	23.0%	77.0%	0.4279	51.27%
U26534	*Nosema apis*	100%	94.0%	0.0584	38.65%
DQ235446	*Nosema apis*	99.0%	94.0%	0.0584	38.69%
U11051	*Nosema necatrix*	100%	96.0%	0.0406	37.05%
AF141129	*Vairimorpha lymantriae*	98.0%	97.0%	0.0314	35.89%
AF033315	*Vairimorpha lymantriae*	100%	97.0%	0.0314	35.38%
HM216911	*Anncaliia algerae*	-	-	0.4675	46.91%
AY894423	*Anncaliia meligethi*	-	-	0.4722	47.07%
EF564602	*Ovavesicula popilliae*	3.0%	94.0%	0.6044	60.95%
AY009115	*Endoreticulatus bombycis*	60.0%	78.0%	0.4222	51.30%
U26532	*Nosema furnacalis*	99.0%	84.0%	0.1937	33.93%
U09282	*Nosema trichoplusiae*	99.0%	83.0%	0.1940	34.09%

"-"No significant similarity found

Type host: Elm leaf beetle, *X. luteola* (Muller) (Coleoptera: Chrysomelidae)

Site of infection: Midgut epithelium of larvae and adults.

Locality: Specimens described here were collected from İstanbul, Turkey.

Transmission: Can occur either horizontally or vertically.

Etymology: The generic epithet "*Rugispora*" derived from the wrinkle structure of exospore. The specific epithet "*istanbulensi*"" refers to Istanbul, where the pathogen was discovered.

Type materials: The samples for light and electron microscopy are preserved in the personal collection of Çağrı BEKİRCAN with the Catalog No. ÇRI-01. *R. istanbulensis*. SSU rRNA gene sequences from the samples were deposited to the GenBank with KR704648 accession code.

Discussion

Up to now, one microsporidium species, *Nosema galerucellae* Toguebaye & Bouix, was described in *X. luteola* from Montpellier, France (Toguebaye and Bouix 1989). That microsporidium carries all the characteristic features of the genus *Nosema* with oval, diplokaryotic spores that measured 4.95 × 2.89 μm mean dimension and have seven to nine coils of the anisofilar polar filament (for other diagnostic characters see Table 3). When *N. galerucellae* is compared with *R. istanbulensis* described here, it differs from *N. galerucellae* and other *Nosema* species in several taxonomic features perhaps most notably the lack of diplokaryotic stages and spores. (Table 3). In addition, spore dimensions are very different between *N. galerucellae* and the new microsporidium. Additionally, according to Sprague et al. (1992) the site of infection is an important taxonomic characteristic. *R. istanbulensis* infected only the intestine while *N. galerucellae* also infects the Malpighian tubules, muscle, fat body, intestine, and trachea. Unfortunately, there is a lack of information on the SSU rRNA gene sequence for *N. galerucellae* to compare the phylogenetic relationships but the described differences in biology and morphology indicate that these microsporidia are not conspecific. On the other hand, when we compared the current microsporidium with *N. bombycis* (type species of *Nosema* genus), there was a high genetic difference (19.4%) between the

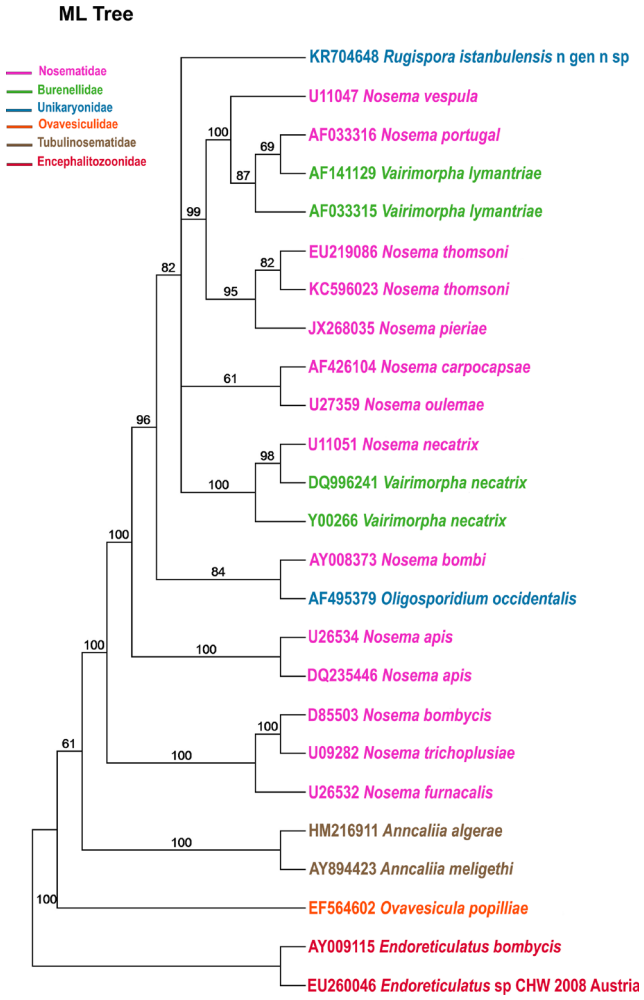

Fig. 4 Maximum likelihood (ML) tree. The topology of the consensus tree was constructed and evaluated by 1000 bootstrap replications. The branches with lower than 50% confidence values were ignored

two taxa according to the phylogenetic distance. These data show that current microsporidium is phylogenetically different from *Nosema* genus.

There are currently five genera of microsporidia where the type species is from a coleopteran hosts: *Anncaliia*, *Canningia*, *Chytridopsis*, *Endoreticulatus*, and *Ovavesicula* (Becnel and Andreadis 2014). *Anncaliia* which is distinct both morphologically and phylogenetically from *R. istanbulensis* and *Chytridiopsis* belongs to the primitive microsporidia (Larsson 2014) and can therefore be excluded. *Ovavesicula* is the microsporidian pathogen of the scarab beetle, and according to molecular, morphological, and developmental features, it differs from the *R. istanbulensis* (Andreadis and Hanula 1987). *Canningia* was described from a bark beetle and all stages are uninucleate and develop in direct contact with the host cytoplasm. There is no molecular data for this genus, and the spore morphology and tissue specificity are distinct from *R. istanbulensis* (Weiser et al. 1995). *Endoreticulatus* was described from a chrysomelid beetle and characterized by having uninucleate stages with spores contained within a parasitophorous vesicle formed by the host endoplasmic reticulum (Brooks et al. 1988). There is no molecular data for the type species but several other described species of *Endoreticulatus* have been sequenced, and they do not appear to be closely related. Microsporidia from beetles have been described from other genera but most are old reports and molecular data is not available. The species with the general characteristics of *R. istanbulensis* and which is phylogenetically related (95% identity, Table 2, Fig. 4) is *Oligosporidium occidentalis* from a mite host (Becnel et al. 2002) Those authors pointed out the discrepancy between the morphological and molecular data (which placed the species with the *Nosema* clade) but found at that time that it is untenable to place a completely uninucleate species in the genus *Nosema* but also questioned placement within the Unikaryonidae (Sprague 1977). The current species poses a similar dilemma where morphological and molecular data are in conflict and most of the time, phylogeny, according to SSU rRNA, is not an enough marker for Microsporidia systematic (O'Mahony et al. 2007; Wang et al. 2015). In addition, the morphological criteria used to define the microsporidian higher taxa

Table 3 Characteristics of the new microsporidium species described from *X. luteola* (Coleoptera: Chrysomelidae) and *N. galerucella*

		Nosema galerucella (Toguebaye ve Bouix, 1989)	*Rugispora istanbulensis* n. gen., n. sp.
Locality		Montpellier, France	Istanbul, Turkey
Host		*Xanthogaleruca luteola* Müller, 1766	*Xanthogaleruca luteola* Müller, 1766
Infected organs		Intestine, malpighian tubules, fat body, muscle, trachea	Intestine
Spore shape		Oval	Oval
Spore size		4.95 × 2.89 μm	3.40 ± 0.37 × 1.63 ± 0.20 μm
Ultrastructural features	Spore wall thickness	80–100 nm	75–115 nm
	Polar filament	7–9	11
	Polar filament diameter	54–69 nm	67–79 nm
	Polaroplast	Lamellar	Lamellar
	Nuclei	Binucleate	Uninucleate

are polyphyletic (Boucias and Pendland 1998). For this reason, the very different taxa, previously identified according to these characters, can come together in phylogenetic trees. Similarly, the current microsporidium came together with some Nosematidae and Burenellidae species in the same group in our cladogram. However, it was different both genetically and morphologically from these species. For instance, there was a fairly SSU sequence difference between current microsporidium and type species of *Vairimorpha* (0.0405–0.0415) similar to *Nosema* species (0.1940). Moreover, the *Vairimorpha* genus is separated from the current microsporidium with morphological characters like a three sporulation sequence and sporophorous vesicle. On the other hand, *Oligosporidium* is the only Unikaryonidae family member associated with the current microsporidium as a result of BLAST analysis in this study. However, the genetic difference between the current microsporidium and *Oligosporidium* is quite enough (0.0527) to separate these taxa.

In conclusion, we decided to assign a new genus (*Rugispora*) of microsporidia for this new species from the elm leaf beetle, *X. luteola*. The pathogen is herein named as *Rugispora istanbulensis* n. gen., n. sp. based on light microscopy, ultrastructural characteristics, and on sequencing of a partial fragment of the SSU rRNA gene.

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