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A new microsporidium, *Vairimorpha subcoccinellae* n. sp. (Microsporidia: Burenellidae), isolated from *Subcoccinella vigintiquatuorpunctata* L. (Coleoptera: Coccinellidae)

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ABSTRACT

A new microsporidium was isolated from *Subcoccinella vigintiquatuorpunctata* L. (Coleoptera: Coccinellidae), a pest of *Galega officinalis* L. in Turkey. Infection in larval and adult stages was systemic with mature spores produced in the midgut, gonads, Malpighian tubules and, most extensively, fat body tissues. The microsporidium was polymorphic with two sporulation sequences producing two types of spores, binucleate spores with 13–15 coils of the polar tube, and uninucleate spores with 7 coils of the polar tube that developed within a sporophorous vesicle (SPV) to form meiospores. The 16S small subunit rRNA (SSU rRNA) gene of the microsporidium was sequenced and compared with twenty-seven microsporidian sequences from GenBank. Based on the phylogenetic analysis of the SSU rRNA sequence, this microsporidium is unique within the *Vairimorpha* group. Morphological and genetic characters indicate that the described microsporidium is dissimilar to all known *Vairimorpha* species, and so is named here as *Vairimorpha subcoccinellae* n. sp.

1. Introduction

Subcoccinella vigintiquatuorpunctata L. is a member of the subfamily Epilachninae (Coleoptera: Coccinellidae). This family of phytophagous coccinellid species are pests of economically important plants (Riddick et al., 2009). *S. vigintiquatuorpunctata* causes damage on different plant species depending on the geographical location (Wheeler and Henry, 1981). In Turkey, larvae and adults of *S. vigintiquatuorpunctata* harm *Galega officinalis* (Fabaceae) by feeding on plant leaves. *G. officinalis* is mainly used medically and for animal nourishment throughout the world (Başaran et al., 2006; Duke, 1987; Lemus et al., 1999).

Microsporidia are eukaryotic obligate pathogenic organisms that infect many different Animalia taxa, especially Insecta (Bekircan et al., 2017a; Canning and Lom, 1986; Solter et al., 2012). This phylum has 200 genera and more than 1300 species (Becnel et al., 2014). They have nonlethal effects on their hosts, including reduced longevity or fecundity, and these tiny organisms can be used as natural regulators against certain insect pest species due to their detrimental effects on their hosts (Hajek and Delalibera, 2010). Because of these effects, microsporidians are also being studied as biological control agents. For instance, *Nosema algerae* (Vavra and Undeen, 1970) reduces the number of malarial oocysts formed in *Anopheles* mosquitoes (Schenker et al., 1992) and *Nosema whitei* (Weiser, 1953) is pathogenic to *Tribolium* (flour beetles) species (Bass and Armstrong, 1992). In addition, *Antonospora locustae* (Slamovits et al., 2004), previously known as *Nosema locustae* (Canning, 1953), is available as a commercial microbial pesticide against grasshoppers and allies (Roberts and Janovy, 2009). Therefore, studies have focused on characterization and description of new microsporidian species in recent years. In this study, a new microsporidian pathogen of *S. vigintiquatuorpunctata* is described based on morphological and molecular data.

2. Materials and methods

2.1. Light microscopy

Larvae and adults of *S. vigintiquatuorpunctata* were collected from April to August 2011–2016 in Ordu, Turkey. The samples were dissected in Ringer's solution and smeared on microscopic slides, then observed under a light microscope at different magnifications (Bekircan et al., 2017b). Infection positive smears were air-dried and fixed in methanol and stained with Giemsa stain (Undeen and Vávra, 1997). Microsporidian spores were photographed with a Nikon Eclipse Ci microscope combined with DS-Fi 2 digital camera. Spore measurements

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

Accession no	Organism name	Host	Order	Family
EU487251	*Vairimorpha* sp. CHW-2008a	*Ocinara lida*	Lepidoptera	Bombycidae
D85503	*Nosema bombycis*	*Bombyx mori*	Lepidoptera	Bombycidae
L39114	*Vairimorpha* sp.	*Bombyx mori*	Lepidoptera	Bombycidae
AY311592	*Vairimorpha* sp. C21	–	–	–
JQ083083	*Vairimorpha* sp. SB-2012	–	–	–
KT698948	*Vairimorpha* sp. Lake Erie	*Manayunkia speciosa*	Polychaeta	Sabellidae
KT698947	*Vairimorpha* sp. Klamath River	*Manayunkia speciosa*	Polychaeta	Sabellidae
Y00266	*Vairimorpha necatrix*	*Pseudaletia unipuncta*	Lepidoptera	Noctuidae
DQ996241	*Vairimorpha necatrix*	*Pseudaletia unipuncta*	Lepidoptera	Noctuidae
KP208681	*Vairimorpha* sp. GB-2014	*Bombyx mori*	Lepidoptera	Bombycidae
D85502	*Vairimorpha* sp. NIS-M12	*Bombyx mori*	Lepidoptera	Bombycidae
AF495379	*Oligosporidium occidentalis*	*Metaseiulus occidentalis*	Acari	Phytoseiidae
EU260046	*Endoreticulatus* sp. *CHW-2008 Austria*	*Thaumetopoea processionea*	Lepidoptera	Thaumetopoeidae
GQ337705	*Vairimorpha* sp. GKK-2009 clone 1	*Agrilus anxius*	Coleoptera	Buprestidae
GQ337707	*Vairimorpha* sp. GKK-2009 clone 3	*Agrilus anxius*	Coleoptera	Buprestidae
U11051	*Nosema necatrix* ATCC 30,460	*Pseudaletia unipuncta*	Lepidoptera	Noctuidae
AF141129	*Vairimorpha lymantriae*	*Lymantria dispar*	Lepidoptera	Erebidae
AF033315	*Vairimorpha lymantriae*	*Lymantria dispar*	Lepidoptera	Erebidae
AJ252955	*Pleistophora ovariae*	*Notemigonus crysoleucas*	Cypriniformes	Cyprinidae
AJ252953	*Pleistophora hippoglossoideos*	*Hippoglossoides platessoides*	Pleuronectiformes	Pleuronectidae
AJ252956	*Pleistophora typicalis*	*Myoxocephalus scorpius*	Scorpaeniformes	Cottidae
JQ082890	*Tubulinosema hippodamiae*	*Hippodamia convergens*	Coleoptera	Coccinellidae
KC412706	*Nosema adaliae*	*Adalia bipunctata*	Coleoptera	Coccinellidae
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
MF037236	*Vairimorpha subcoccinellae* n. sp.	*Subcoccinella vigintiquatuorpunctata*	Coleoptera	Coccinellidae

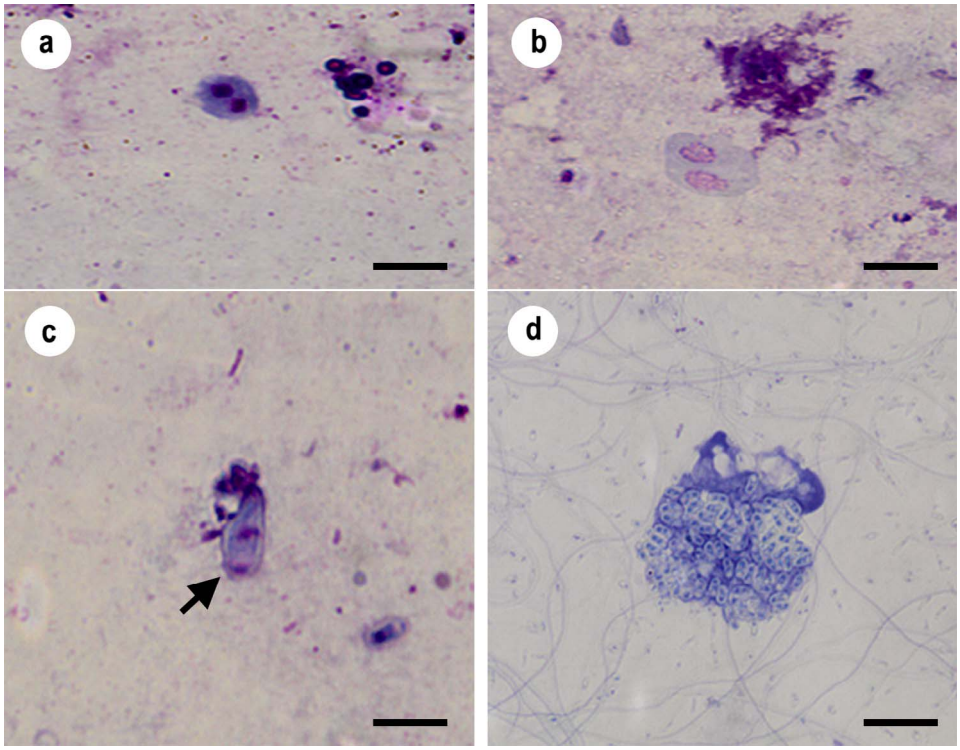

Fig. 1. Light microscopy of the Giemsa stained stages of *Vairimorpha subcoccinellae* n. sp life cycle. a: Spherical binucleate meront (Gut); b: Binucleate sporont (Gut); c: Sporoblast (Gut), (Unite bars = 1 μm); d: Mature spores (Malpighian tubules and gonads), (Unite bar = 2 μm).

were taken using Nikon NIS Elements imaging software.

2.2. Electron microscopy

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). A Leica EM UC7 ultramicrotome was used to make thin sections, and these were 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 JEOL JEM 1010 transmission electron microscope.