DOI: 10.1515/ap-2016-0032

Acta Parasitologica, 2016, 61(2), 228–231; ISSN 1230-2821

DE GRUYTER

First report and spore ultrastructure of *Vairimorpha plodiae* (Opisthokonta: Microspora) from *Plodia interpunctella* (Lepidoptera: Pyralidae) in Turkey

Mustafa Yaman[1,4]*, F. Pınar Güngör[1], Beyza Gonca Güner[1], Renate Radek[2] and Andreas Linde[3]

[1]Department of Biology, Faculty of Science, Karadeniz Technical University, 61080, Trabzon, Turkey;
[s2]Free University of Berlin, Institute of Biology/Zoology, Königin-Luise-Str. 1–3, 14195 Berlin, Germany;
[3]University for Sustainable Development, Forest and Environment, Applied Ecology and Zoology, Alfred-Möller-Str. 1, 16225 Eberswalde, Germany; [4]Faculty of Education, Ordu University, Ordu, 52200, Turkey

Abstract

The present study describes the first isolation and characterization of *Vairimorpha plodiae*, a microsporidian pathogen of *Plodia interpunctella* (Lepidoptera: Pyralidae), from Turkey. We present characteristic light and electron microscopical features of the spores. Fresh binucleate spores are oval and measure 4.48 ± 0.23 (4.01–4.84) µm in length and 2.21 ± 0.15 (1.91–2.48) µm in width. Ultrastructural studies showed that the spore wall measures 150 to 200 nm and consists of a clear endospore (125–150 nm) and an electron-dense, uniform, thin exospore (30–50 nm). The polar filament is isofilar and with 10–12 coils. The well-developed polaroplast consists of two parts with thin lamellae anteriorly and thick, irregularly arranged lamellae posteriorly. The identity of our isolate is discussed.

Keywords

Plodia interpunctella, *Vairimorpha plodiae*, microsporidium, biological control, spore morphology

Introduction

The Indian-meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae), is one of the most common pests in stored products such as dried fruits, nuts and flour not only in Turkey but in the whole world (Malone 1984; Kıvan and Karsavuran 1991; Turanlı 2003; Grieshop 2005). Chemical control strategies have been of limited use because of undesirable effects: Non-target organisms including useful insects, man and environment may be harmed, resistance against the pesticides may develop, and remaining toxic residues on stored products pose a possible threat for consumers (FAO 1992). Several alternative control measures have been used so far to prevent the increased spreading of this pest and economical loses (Malone 1984; Tunc *et al*. 2000; Ayvaz *et al*. 2008, 2010). The most promising results for the control of this pest were reported with the use of natural enemies (Schöller and Filinn 2000; Grieshop 2005; Shojaaddini *et al*. 2012). Entomopathogens such as microporidia can be potential biological control agents of insects. Most microsporidian species are very host specific avoiding non-target effects on other insect species.

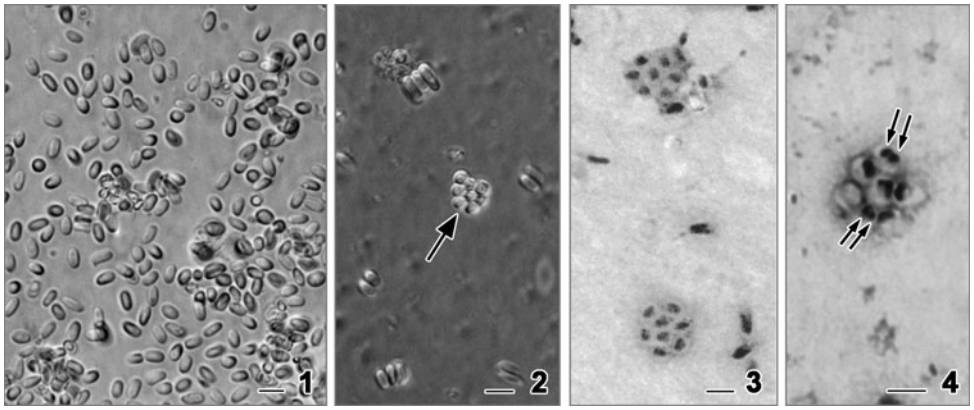
Kellen and Lindegren (1968, 1969) described two microsporidian species from *P. interpunctella* in California, *Nosema plodiae* and *Thelohania nana*. Maddox and Sprenkel (1978) and Malone and Canning (1982) later concluded that those two species were in fact a single, dimorphic species which should be considered a member of the genus *Vairimorpha*. To date, no microsporidia have been found in *P. interpunctella* in Turkey.

Here, we provide the characteristic features and occurrence of a Turkish isolate of *Vairimorpha plodiae* and present details on the ultrastructure of the spores.

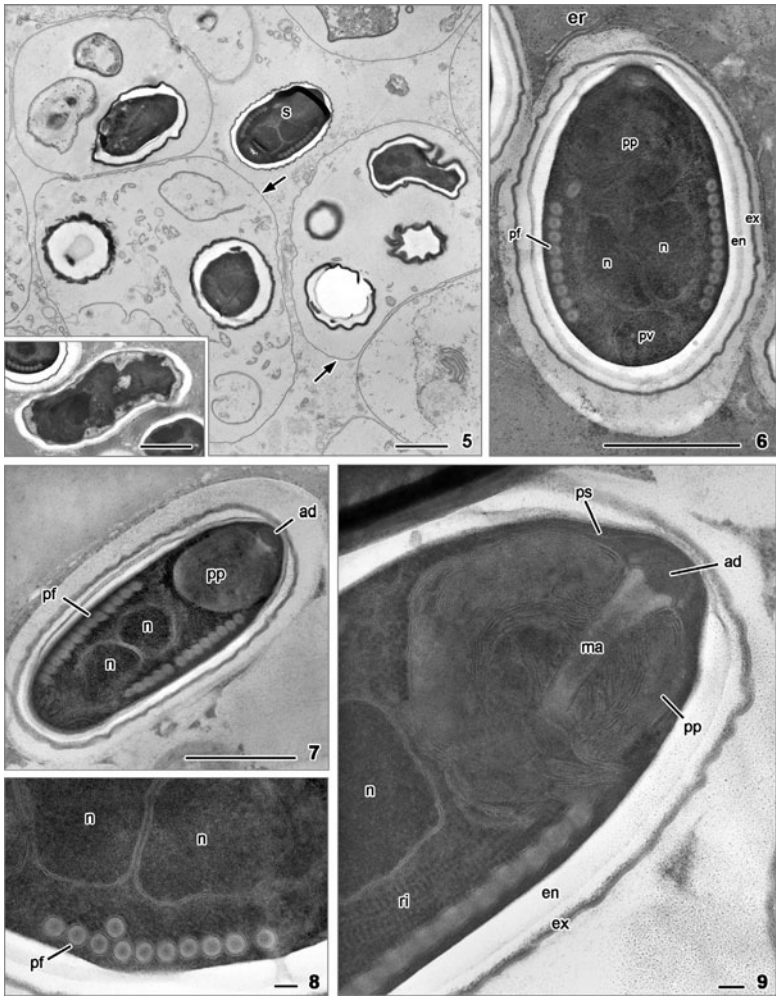
Materials and Methods

Four hundred and twenty eight *P. interpunctella* larvae were collected from different stored products such as hazelnut, rice, apricot and fig in Trabzon, Turkey. The larvae were dissected in Ringer's solution and wet smears of tissue samples were examined under a microscope for identification of pathogens. When an infection was observed, a part of the material was

*Corresponding author: muyaman@hotmail.com



Figs 1–4. Light micrographs of the spores of *Vairimorpha plodiae* from *Plodia interpunctella* in fresh and Giemsa-stained smears. 1. Fresh free spores. 2–4. Sporophorous vesicles containing 8 spores (octospores, large arrow). Double arrows = binucleated spores. Bars = 5 μm



Figs 5–9. Transmission electron micrographs of *Vairimorpha plodiae* from *Plodia interpunctella*. 5. Sporophorous vesicles, s; free spore (Bar = 1 μm), arrows; wall of sporophorous vesicle. Inset: Sporoblast with wall consisting of thin electron lucent and electron dense layers (Bar = 1 μm). 6, 7. Longitudinal sections of spores (Bar = 1 μm). 8. Longitudinal section of posterior part of a spore showing 10– 12 coils of the polar filament and a clearly visible diplokaryon (Bar = 100 nm). 9. Section of the anterior portion of a spore showing an anchoring disc and a well-developed, thin lamellae-type polaroplast. ad, anchoring disc, en; endospore, ex; exospore, ma; manubrium, n; nucleus, pf; polar filament, pp; polaroplast, ps; polar sac, pv, posterior vacuole, ri; ribosomes (Bar = 100 nm)

used for preparation of Giemsa-stained smears and another part was used for ultrastructural studies. Fresh and stained spores of microsporidia were measured and photographed using an Olympus BX51 microscope with a DP-25 digital camera and a DP2-BSW Soft Imaging System.

For transmission electron microscope studies, portions of infected tissues were prepared using standard protocols (Yaman and Radek 2003, Yaman *et al*. 2008). Thin sections were examined with a Philips EM 208 transmission electron microscope (TEM).

Results

A microsporidian infection was found in larvae of *P. interpunctella* collected in Trabzon (Turkey). Light microscopic examinations of the parasitized individuals revealed the presence of the pathogen in a variety of tissues and organs including salivary glands, midgut, Malpighian tubules, ovaries and fat bodies. Two types of spores were found: Free binucleate spores and sporophorous vesicles containing eight uninucleate spores (octospores). Fresh binucleate spores are oval, 4.48 ± 0.23 (4.01–4.84) µm in length and 2.21 ± 0.15 (1.91–2.48) in width (n = 50). Smaller uninucleate spores in sporophorous vesicles are 3.48 ± 0.33 µm in length and 2.48 ± 0.36 µm in width (n = 50), and sporophorous vesicles are 11.2–13.5 µm in diameter. Giemsa stained binucleate spores are 3.89 ± 0.31 (3.06–4.45) µm in length and 2.19 ± 0.17 (1.70–2.38) in width (n = 50). Ultrastructural studies showed that the free, oval spores contain two nuclei in diplokaryotic arrangement. The nuclei measure 500–600 nm in diameter (Figs 5, 7, 8). The spore wall is thick and measures 150 to 200 nm and consists of a clear endospore (125 to 150 nm) and an electron-dense, uniform, thin exospore (30–50 nm) (Figs 6–9). The polar filament is isofilar, relatively thin (80–100 mm)and has 10–12 coils (Figs 7, 8). The well-developed polaroplast consists of two lamellated parts – thin lamellae anteriorly and thick, irregularly arranged lamellae posteriorly with (Fig. 9).

Discussion

The protist pathogen found in *P. interpunctella* is a microsporidium. The spore ultrastructure of the binucleate spore elucidated the typical characteristics of microsporidia such as a polar filament with an anchoring disc, a polaroplast, a posterior vacuole and a lack of mitochondria (Figs 7–9) (Larsson 1986, 1988, 1999; Canning and Vavra 2000). The light microscopic and ultrastructural studies on the production of two spore types suggest that it belongs to the genus *Vairimorpha*. The genus *Vairimorpha* is characterized by a predominantly disporoblastic development producing free, binucleate spores and an octosporoblastic sequence producing smaller uninucleate spores in a sporophorous vesicle (Malone 1984). During the study, we observed both free, binucleate spores and sporophorous vesicles including eight spores. To date, one species from the genus *Vairimorpha, V. plodiae* is known from *P. interpunctella*. The species was first described as a mixed infection of *Nosema plodiae* (Kellen and Lindegren 1968) and *Thelohania nana* (Kellen and Lindegren 1969). Later the genus name was corrected and the species renamed as *Vairimorpha plodiae* (Malone and Canning 1982).

The tissue specificity of our microsporidian isolate from *P. interpunctella* is very identical to that described in the literature (Kellen and Lindegren, 1968; Malone, 1984). The spore dimension is a suitable morphological character to distinguish pathogens or their different geographic isolates. Our isolate of *V. plodiae* has larger spores (4.48 ± 0.23 × 2.21 ± 0.15 µm) than in the original description (4.09 ± 0.24 µm × 1.89 ± 0.74 µm) (Kellen and Lindegren, 1968) but smaller spores than the isolate described by Malone (1984) (5.12 ± 0.05 µm × 2.90 ± 0.03 µm). The spores Malone (1984) received from a laboratory culture of *Galeria mellonella* infected with his isolate of *V. plodiae* were also larger (4.86 ± 0.03 µm × 2.60 ± 0.02 µm) than our Turkish isolate .

The ultrastructural characteristics of the spore are important features for comparison of species of microsporidian species (Larsson 1986, 1988, 1999; Canning and Vavra 2000; Yaman *et al*. 2005; 2011). The ultrastructure of many *Vairimorpha* species has been described (Canning *et al*. 1999; Vavra *et al*. 2006), but to date there is no record on the ultrastructural characteristics of *V. plodiae* spores. The original description of *N. plodiae* from *P. interpunctella* was based solely on light microscopy (Kellen and Lindegren 1968). Malone and Canning (1982) studied the fine structures of life cycle stages of *V. plodiae* including meront, sporont and sporoblast in detail, but could not present details on the ultrastructure of mature spores. Here, the ultrastructural feature of the mature spores from *V. plodiae* are presented and compared with other *Vairimorpha* species infecting lepidopteran pests. The free, binucleate spore of *V. plodiae* has an isofilar polar filament with 10–12 coils. This number is different from the number of coils of other *Vairimorpha* species from lepidopteran hosts, such as *V. necatrix* (10–15 coils) (Maddox and Sprenkel, 1978) and *V. ephestiae* (14–16 coils) (Weiser and Purrini 1985) and resembles *V. disparis* (11–13 coils) (Vavra *et al*. 2006).

The structural features of our microsporidian isolate from *Plodia interpunctella* identify it as *Vairimorpha plodiae*. This is the first microsporidian record from *P. interpunctella* in Turkey, and also the first of the genus *Vairimorpha* in Turkey.

It is often difficult to compare microsporidian species descriptions because early descriptions were based mainly on light microscopic spore morphology; ultrastructural details were not available. These restricted methods resulted in the unnecessary creation of new species. Our findings presented here will be useful for the identification and comparison of other *Vairimorpha* species from lepidopteran hosts.

References

Ayvaz A., Albayrak S., Karaborklu S. 2008. Gamma radiation sensitivity of the eggs, larvae and pupae of Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). *Pest Management Science*, 64, 505–512. DOI:10.1002/ps.1526

Ayvaz A., Sagdic O., Karaborklu S., Ozturk I. 2010. Insecticidal activity of the essential oils from different plants against three stored-product insects. *Journal of Insect Science*, 10, 1–13. DOI:10.1673/031.010.2101

Canning E.U., Curry A., Cheney S., LaFranchi-Tristem N.J., Haque M.A. 1999. *Vairimorpha imperfecta* n. sp., a microsporidian exhibiting an abortive octosporous sporogony in *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). *Parasitology*, 119, 273–286. DOI: 10.1017/S0031182099004734

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

FAO, 1992. Pesticide residues in food. Report no. 116. pp. 146

Grieshop M.J. 2005. Evaluation of three species of *Trichogramma* egg parasitoids for biological control of the Indian meal moth in warehouses and retail stores. Ph.D. Thesis, Kansas State University, pp. 227

Kellen W.R., Lindegren J.E. 1968. Biology of *Nosema plodiae* sp. n., a microsporidian pathogen of the Indian-meal moth, *Plodia interpunctella* (Hübner), (Lepidoptera:Phycitidae). *Journal of Invertebrate Pathology*, 11, 104–111. DOI: 10.1016/0022-2011(68)90059-1

Kellen W.R., Lindegren J.E. 1969. Host-pathogen relationships of two previously undescribed microsporidia from the Indian-meal moth, *Plodia interpunctella* (Hübner), (Lepidoptera: Phycitidae). *Journal of Invertebrate Pathology*, 14, 328–335. DOI: 10.1016/0022-2011(69)90159-1

Kıvan M., Karsavuran Y. 1991. Effect of the food on the larval development of *Plodia interpunctella* (Hübner), (Lepidoptera:Phycitidae). *Turkish Journal of Entomology*, 15, 113–116 (In Turkish)

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. *Archiv für Protistenkunde*, 136, 1–37.

Larsson J.I.R. 1999, Identification of microsporidia. *Acta Protozoologica*, 38**,** 161–197

Maddox J.V., Sprenkel R. K. 1978. Some enigmatic microsporidia of the genus *Nosema*. *Misc. Publ. Entomol. Soc. Am.*, 11:65–84

Malone L.A., Canning E.U. 1982. Fine structure of *Vairimorpha plodiae* (Microspora: Burenellidae), a pathogen of *Plodia interpunctella* (Lepidoptera: Phycitidae) and infectivity of the dimorphic spores. *Protistologica*, 18, 503–516

Malone L.A. 1984. A comparison of the development of *Vairimorpha plodiae* and *Vairimorpha necatrix* in the Indian meal moth, *Plodia interpunctella*. *Journal of Invertebrate Pathology*, 43, 140–149. DOI:10.1016/0022-2011(84)90131-9

Schöller M., Flinn P.W. 2000. Parasitoids and predators. In: Subramanyam, B., Hagstrum, D.W. (Eds.), Alternatives to Pesticides in Stored-product IPM. *Kluwer Academic Publishers*, Norwell, MA, pp. 229–271

Shojaaddini M., Lopez M.J., Moharramipour S., Khodabandeh M., Talebi A. A., Vilanova C., Latorre A. and Porcar M. 2012. A *Bacillus thuringiensis* strain producing epizootics on *Plodia interpunctella*: A case study. *Journal of Stored Products Research*, 48, 52–60. DOI: 10.1016/j.jspr.2011.09.003

Tunc I., Berger B.M., Erler F., Dagli F. 2000. Ovicidal activity of essential oils from five plants against two stored-products insects. *Journal of Stored Products Research*, 36:161–168. DOI: 10.1016/S0022-474X(99)00036-3

Turanlı F. 2003. Studies on infestation levels of pests on dried fig in Aydın and İzmir Provinces. *Turkish Journal of Entomology*, 27, 171–180 (In Turkish)

Vavra J., Hylis M., Vossbrinck C.R., Pilarska D., Linde A.,Weiser J., McManus M. L., Hoch G., Solter L. F. 2006. *Vairimorpha disparis* n. comb. (Microsporidia: Burenellidae): a redescription and taxonomic revision of *Thelohania disparis* Timofejeva 1956, a microsporidian parasite of the gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae). *Journal of Eukaryotic Microbiolology*, 53, 292–304. DOI: 10.1111/j.1550-7408.2006.00108.x

Weiser J., Purrini K. 1985. Light and electron microscopic studies on the microsporidian *Vairimorpha ephestiae* (Mattes) (Protozoa, Microsporidia) in the meal moth *Ephestia kuhniella*. *Archiv für Protistenkunde*, 130, 179–189

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

Yaman M., Radek R., Aslan I., Ertürk Ö. 2005. Characteristic features of *Nosema phyllotretae* Weiser 1961, a microsporidian parasite of *Phyllotreta atra* (Coleoptera: Chrysomelidae) in Turkey. *Zoological Studies*, 44, 368–372. DOI: 10.1007/s10340-005-0100-y

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

Yaman M., Özcan N., Radek R., Linde A., Lipa J.J. 2011. Ultrastructure, characteristic features and occurrence of *Nosema leptinotarsae* Lipa 1968, a microsporidian pathogen of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Acta Parasitologica*, 56, 1–7. DOI: 10.2478/s11686-011-0009-y

Received: April 9, 2015
Revised: September 10, 2015
Accepted for publication: November 27, 2015