

## A New Microsporidian Parasite Record of *Phyllotreta undulata* (Chrysomelidae, Coleoptera)

Mustafa YAMAN

Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, 61080, Trabzon - TURKEY

Renate RADEK

Institute of Biology/Zoology, Free University of Berlin, Königin-Luise-Str. 1-3, 14195 Berlin - GERMANY

Received: 04.03.2004

**Abstract:** A new microsporidian parasite of *Phyllotreta undulata* was recorded in Turkey. Fresh spores are oval,  $3.85 \pm 0.69 \mu\text{m}$  (2.0-4.75) in length and  $2.04 \pm 0.23 \mu\text{m}$  (1.90-2.40) in width ( $n = 50$ ). The spores are uninucleated. As there were insufficient data available on the complete life cycle of the parasite, it could not be identified at species level.

**Key Words:** microsporidian parasite, *Phyllotreta undulata*, Chrysomelidae

### Yeni Bir *Phyllotreta undulata* (Coleoptera, Chrysomelidae) Paraziti Kaydı

**Özet:** Bu çalışmada *Phyllotreta undulata* için parazitik yeni bir mikrosporidium kaydedilmiştir. Parazite ait sporlar oval,  $3.85 \pm 0.69 \mu\text{m}$  (2.0-4.75) ve  $2.04 \pm 0.23 \mu\text{m}$  (1.90-2.40) ebatlarında olup tek nükleusludur. Parazitin yaşam döngüsünün tamamı hakkında yeterli bilgi olmadığı için, parazit tür seviyesinde tanımlanamamıştır.

**Anahtar Sözcükler:** Microsporidia paraziti, *Phyllotreta undulata*, Chrysomelidae

### Introduction

The first microsporidian described from Chrysomelidae was *Nosema phyllotretae* Weiser, observed in *Phyllotreta atra* (F.) and *Phyllotreta undulata* (Kutschera) (Weiser, 1961; Toguebaye et al., 1988), and it is the sole microsporidium reported from *P. undulata*. In the present paper we report a new microsporidian parasite of *P. undulata*.

### Materials and Methods

Adult *P. undulata* specimens were collected from the central and east Black Sea regions of Turkey in 2001. Each beetle was dissected and wet smears were examined under a light microscope. Detected spores were measured and photographed and sections of infected beetles were examined with a Philips 208 electron microscope.

For ultrastructural studies in a transmission electron microscope (TEM) different portions of infected beetles 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  $\text{OsO}_4$  according to Karnovsky (a fresh

1:1 mixture of 2%  $\text{OsO}_4$  and 3%  $\text{K}_4[\text{Fe}(\text{CN}_6)]$ ) for 1.5 h, rinsed in cacodylate buffer and dehydrated in ethanol prior to embedding in Spurr's resin (Spurr, 1969). Thin sections were mounted on Pioloform-coated copper grids stained with saturated uranyl acetate and Reynolds' lead citrate (Reynolds, 1963). They were examined in a Philips 208 TEM.

### Results and Discussion

A microsporidian infection was found in the adults of *P. undulata* collected from Beyyence (Çarşamba-Samsun). Microscopic examination of parasitized individuals revealed the presence of the parasite in Malpighian tubules. The spores were only observed in this tissue (Figure 1). Spores were in groups. Fresh spores are oval,  $3.85 \pm 0.69 \mu\text{m}$  (2.0-4.75) in length and  $2.04 \pm 0.23 \mu\text{m}$  (1.90-2.40) in width. The spore is uninucleate. The polar filament of the parasite has 6-7 coils, and the well-developed polaroplast was of the lamellated type with irregularly arranged membranes anteriorly and roughly parallel cisternae posteriorly (Figure 2).



Figure 1. The spores of the microsporidian parasite in Malpighian tubule (1000x).

The spore's ultrastructure elucidated typical characteristics of Microsporidia such as polar filament, an anchoring disc and lack of mitochondria. Microsporidia, known to infect *Chrysomelidae* are included in 3 genera: *Nosema* Naegeli, 1857, *Unikaryon* Canning, Lai and Lie, 1974 and *Pleistophora* Gurley, 1893 (Toguebaye et al., 1988). However, Brooks et al. (1988) described a new genus, *Endoreticulatus*, for the microsporidium *Pleistophora fidelis* (Hostounsky and Weiser, 1975), based on the ultrastructure of a microsporidium in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Microsporidians of the genus *Nosema* are diplokaryotic at all developmental stages and disporous, while the genera *Unikaryon* and *Endoreticulatus/Pleistophora* are monokaryotic at all stages (Toguebaye et al., 1988). The spore is the most important life cycle stage for the identification of microsporidia above the species level by ultrastructural studies. The spore is always present and provides abundant characters to evaluate (Larsson, 1999). The ultrastructure of spores of the genus *Nosema* was given by Canning and Vavra (2000) and Yaman and Radek (2003). The genus *Nosema* has been used to collect species with diplokaryotic spores (Larsson, 1999). As seen in Figure 2, the present microsporidian is

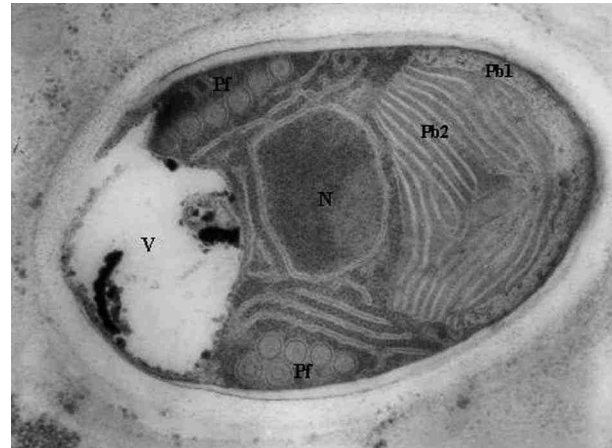


Figure 2. Longitudinal section of the microsporidian spore. P1, anterior part and P2, posterior part of polaroplast; N, nucleus; Pf, polar filament; V, posterior vacuole (32,000x)

uninucleate. The spore dimension is a good feature for comparison of the microsporidia from chrysomelids. This microsporidian isolate (3.85 x 2.04  $\mu\text{m}$ ) differs from *Nosema phyllotretae* (4.2 to 6 x 2 to 3  $\mu\text{m}$ ) in spore size. Furthermore, the present microsporidium infected only Malpighian tubules. Therefore, the parasite characterized here is considered a new microsporidian parasite of *Phyllotreta undulata*. Further research will be directed to observe other life stages of the parasite and identify it at the species level.

### Acknowledgments

Mustafa Yaman was awarded a grant within the scope of the NATO Science Fellowship Program by the Scientific and Technical Research Council of Turkey (TÜBİTAK) (B.02.1.BAK.0.09.00.00 (2001)/477/1191) for this study. The author wishes to express his thanks to Prof. Dr. Klaus Hausmann for providing the necessary laboratory space and advanced training in electron microscopy of entomopathogenic protists (Institute of Biology/Zoology, Free University of Berlin, Königin-Luise-Str. 1-3, 14195 Berlin, Germany). The authors are grateful to Assoc. Prof. Dr. Irfan Arslan for identifying the insect.

## References

- Brooks, W.M., Becnel, J.J. and Kennedy, G.G. 1988. Establishment of *Endoreticulatus* n. g. for *Pleistophora fidelis* (Hostounsky and Weiser, 1975) based on the ultrastructure of a microsporidium in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). J. Protozool. 35: 481-488.
- Canning, E.U. and Vavra, J. 2000. Phylum Microsporidia. In: The Illustrated Guide to the Protozoa (eds., Lee, Leedale and Bradbury), Allen Press Inc., Lawrence, pp. 39-126.
- Hostounsky, Z. and Weiser, J. 1975. *Nosema polygrammae* sp. n. and *Pleistophora fidelis* sp. n. (Microsporidia, Nosematidae) infecting *Polygramma undecimlineata* (Coleoptera: Chrysomelidae) in Cuba. Vest. Cs. Spol. zool. 39:104-110.
- Larsson, J.I.R. 1999. Identification of Microsporidia. Acta Protozool., 38: 161-197.
- 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.
- Spurr, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. Clin. Microbiol. Res., 3:197-218.
- Toguebaye, B.S., Marchand, B. and Bouix, G. 1988. Microsporidia of Chrysomelidae. In: Petitpierre, E., Hsiao, T. H., Jolivet, P. H. (Eds.), Biology of Chrysomelidae, Kluwer Academic Publishers, Boston, pp. 399-416.
- Weiser, J. 1961. Die mikrosporidien als parasiten der insekten. Monogr. Angew. Entomol. 17: 1-149.
- Yaman, M. and Radek, R. 2003. *Nosema chaetocnema* sp. n., a microsporidian (Microspora; Nosematidae) parasite of *Chaetocnema tibialis* (Chrysomelidae, Coleoptera). Acta Protozoologica 42: 231-237.